

CORRELATION BETWEEN LEVELS OF SULCULAR AND CAPILLARY BLOOD GLUCOSE IN SCREENING OF DIABETES MELLITUS IN CHRONIC PERIODONTITIS PATIENT

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**BRANCH – II
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CERTIFICATE

This is to certify that **Dr.HARRY NIHAL NAYAGAM**, Post Graduate student (2008-2011) in the Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, has done this dissertation titled **CORRELATION BETWEEN LEVELS OF SULCULAR AND CAPILLARY BLOOD GLUCOSE IN SCREENING OF DIABETES MELLITUS IN CHRONIC PERIODONTITIS PATIENT** under our direct guidance and supervision in partial fulfillment of the regulations laid down by the **Tamil Nadu Dr.M.G.R. Medical University**, Chennai - 600 032 for **M.D.S., (Branch-II) Periodontology** degree examination.

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ABSTRACT

BACKGROUND

Diabetes mellitus is a heterogeneous metabolic disease characterized by impaired glucose tolerance and altered carbohydrate and lipid metabolism. The number of diabetics in Indian population is increasing at an alarming pace, the WHO has predicted that by 2025, about 37 million people will be diabetics. Diabetes mellitus remains undiagnosed in approximately 50% of the patients actually suffering from the disease. Importantly, the prevalence of diabetes in periodontitis patients is twice as high when compared to periodontally healthy subjects. Hence, a large number of patients with periodontitis may have undiagnosed diabetes mellitus.

AIM OF THE STUDY

To evaluate the correlation between gingival sulcular blood glucose and capillary blood glucose level for screening of diabetes mellitus in chronic periodontitis patients.

MATERIALS AND METHOD

30 diabetic and 30 non-diabetic subjects with moderate to severe chronic periodontitis were enrolled and subjected to routine periodontal examination. Blood oozing from the gingival sulcus is collected using a micropipette from the gingival crevice of maxillary anterior teeth and transported to a test-strip of a self monitoring glucometer (Accu-check Active). Finger-prick capillary blood glucose levels values are considered as the control. The data was statistically analyzed using Mann-Whitney U-test, Wilcoxon Signed Ranks test and Spearman's Rank correlation coefficient test.

RESULTS

The blood glucose values by both the methods i.e, gingival crevicular and finger-prick capillary are highly positively significantly correlated ($p < 0.0001$). Also in both the groups, the reliability of the two procedures is significantly high ($p < 0.0001$).

CONCLUSION

The results obtained from this study suggests that blood expressed during routine periodontal examination may be used as a rapid chair-side screening procedure for diabetes mellitus in a dental office setting.

DECLARATION

TITLE OF DISSERTATION	CORRELATION BETWEEN LEVELS OF SULCULAR AND CAPILLARY BLOOD GLUCOSE IN SCREENING OF DIABETES MELLITUS IN CHRONIC PERIODONTITIS PATIENT
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Signature of the candidate

CONTENTS

Sl. No	TITLE	Page No
1	INTRODUCTION	1
2	AIM AND OBJECTIVES	3
3	REVIEW OF LITERATURE	4
4	MATERIALS AND METHOD	25
5	RESULTS	44
6	DISCUSSION	62
7	SUMMARY AND CONCLUSION	69
8	BIBLIOGRAPHY	71
9	APPENDIX : PROFORMA AND INFORMED CONSENT	80

LIST OF PHOTOGRAPHS

Photo No	Title	Pg.No
1	Orthopantomogram	38
2	Chronic periodontitis	38
3	Clinical attachment loss of 5mm	39
4	Armamentarium for clinical examination	39
5	Micropipette	40
6	Glucometer, lancet, test-strip	40
7	Micropipette used to collect sulcular blood	41
8	Sulcular blood being transferred to the test-strip and the blood glucose reading on the glucometer monitor	41
9	Swabbing the pad of the index finger with surgical spirit	42
10	The lancet in position on the index finger	42
11	A drop of blood on the pad of the index finger	43
12	Glucometer showing reading	43

LIST OF TABLES

Table No.	Title	Pg.No
1	Group I : Type 2 diabetic subjects with chronic periodontitis - Master chart	48
2	Group II : Non - diabetic subjects with chronic periodontitis - Master chart	49
3	Comparison of mean values between two groups.	50
4	Comparison of mean values between 2 methods in group 1.	51
5	Comparison of mean values between the 2 methods in group II	52
6	Results of correlation analysis between gingival and capillary methods in both group I and group II.	53
7	Results of reliability analysis in both group I and group II	54

LIST OF FIGURES

Figure No	Title	P.No
1	Mean values of finger-prick capillary blood glucose level and gingival sulcular blood glucose level in diabetic and non-diabetic groups.	55
2	Comparison of mean values between 2 methods in group 1	56
3	Comparison of mean values between the 2 methods in group II	57
4	Comparison of gingival crevicular blood glucose level and capillary blood glucose level in diabetic subjects with chronic periodontitis.	58
5	Comparison of gingival crevicular blood glucose level and capillary blood glucose level in non-diabetic subjects with chronic periodontitis.	59
6	Scattered diagram to estimate the correlation between capillary blood glucose level and gingival crevicular blood glucose level in group 1 subjects.	60
7	Scattered diagram to estimate the correlation between capillary blood glucose level and gingival crevicular blood glucose level in group II subjects.	61

LIST OF ABBREVIATIONS

AAP	–	American Academy of Periodontology
ADA	–	American Diabetes Association
AGE	–	Advanced Glycation End-products
BOP	–	Bleeding on Probing
CBGL	–	Capillary Blood Glucose Level
DM	–	Diabetes mellitus
Ed	–	Edition
GCB	–	Gingival Crevicular Blood
GCF	–	Gingival Crevicular Fluid
GOD	–	Glucose oxidase
HbA1c	–	Glycosylated Hemoglobin Assay
IDDM	–	Insulin dependent diabetes mellitus
IVGTT	–	Intravenous Glucose Tolerance Test
IL - 1 β	–	Interleukin -1 beta
LCD	–	Liquid Crystal Display.
mm	–	Millimeter
NAD	–	Nicotinamide adenine dinucleotide
NADH	–	Nicotinamide adenine dinucleotide dehydrogenase
NIDDM	–	Non-insulin dependent diabetes mellitus.
PMN	–	Polymorpho nuclear leucocytes
PPD	–	Probing pocket depth
SMBG	–	Self monitoring blood glucose

INTRODUCTION

Diabetes mellitus encompasses a heterogeneous group of disorders with the common characteristic of altered glucose tolerance or impaired lipid and carbohydrate metabolism (*Mealey B et al, 1999*⁷⁰). Diabetes mellitus develops from either a deficiency in insulin production or an impaired utilization of insulin. Based upon these two conditions, diabetes mellitus can be divided into 2 main types - Type I (formerly Insulin Dependent Diabetes Mellitus) and Type II (formerly Non-Insulin dependent Diabetes Mellitus).

The World Health Organization has recently acknowledged that India has the maximum number of diabetic patients than any other country (around 35 million). This is projected to increase to 57 million by the year 2025. The prevalence has increased from 0.6% in 1975 to 2.4% in 1995 (*Kokiwar et al, 2007*⁶¹). The prevalence of periodontitis is significantly greater in diabetic subjects than in non-diabetic individual (*Cianciola LJ, Park BH et al, 1982*¹⁷). Among diabetic individuals, those with poorer metabolic control have often been found to have severe periodontal destruction.

Gingival bleeding is an early manifestation of periodontal disease and in diabetics gingival bleeding is even more severe (*Ervash T et al, 1985*²⁹). The sulcular blood that is expressed during routine periodontal examination can be used as an excellent alternative source of blood for glucometric analysis using the technology of portable glucometers (self monitoring blood glucometer) (*Stein GM et al 1969*¹⁰¹, *Parker RC et al, 1993*⁹⁴, *Beikler et al 2002*¹⁰, *Ardakani et al 2009*⁶).

This method of glucometric analysis was selected because, with this method, dentists are relatively more comfortable in obtaining blood samples, the patient is not unduly

alarmed, and there is no fear complex involved for the patient. Though diabetes mellitus is one of the most common metabolic disorder, nearly half the cases go undiagnosed (*Hadden WC, Harris MI et al, 1987*⁴³). Patients with undiagnosed diabetes mellitus are at significantly increased risk for coronary heart disease, stroke and peripheral vascular disease (*Expert Committee on Diagnosis and Classification of Diabetes Mellitus, 1997*³⁰).

Due to the close association between diabetes and periodontitis, it can be assumed that the dental practitioner and periodontists are extremely likely to encounter an increasing number of undiagnosed diabetes patients with periodontitis. The early diagnosis of diabetes can help to prevent its long-term complications that are responsible for the high morbidity and mortality of diabetes patients (*Harris MI, 2000*⁴⁵).

It is essential for a patient suffering from diabetes mellitus to regularly monitor the concentration of blood glucose in order to prevent any unseen complications that may occur. Thus, development of a non-invasive assay for biochemical materials is an urgent necessity and it will allow us to utilize these screening tests widely for many patients.

Hence the present study was undertaken to establish the role of a dentist in screening patients with undiagnosed diabetes with the help of a relatively comfortable, non-invasive and chair-side technique during routine periodontal examination. With improved screening and diagnostic tools it can be expected that an increasing percentage of population will be diagnosed as diabetics.

AIM AND OBJECTIVES

Aim of the study:

To evaluate the correlation between gingival sulcular blood glucose and capillary blood glucose level for screening of diabetes mellitus in chronic periodontitis patients.

Objective of the study:

1. To evaluate the blood glucose level using the gingival sulcular blood and capillary blood method.
2. To compare the mean difference in glucose levels between the gingival sulcular blood and capillary blood.

REVIEW OF LITERATURE

Diabetes mellitus is a disease of metabolic dysregulation, primarily of carbohydrate metabolism, characterized by hyperglycemia that results from defects in insulin secretion, impaired glucose function or both. (*Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003*⁹¹).

Type I diabetes mellitus is caused by cell-mediated autoimmune destruction of the insulin-producing beta cells of the islets of Langerhans in the pancreas. Type II diabetes mellitus is caused by peripheral resistance to insulin action, impaired insulin secretion and glucose production in the liver (*Atkinson M A, Eisenbarth G S, 2001*⁸).

Chronic periodontitis, an infectious disease that causes destruction of the supporting structures of the teeth, can be modified by systemic conditions like diabetes mellitus to an extent that it is now its sixth complication (*Loe H, 1993*⁶⁶).

The prevalence of diabetes mellitus is on the rise and its influence on periodontal disease suggests that it is likely to become an integral part of the patient population and both general dentists and periodontists will have to manage such patients (*Lawrence S Miller, Mary Agnes et al, 1992*⁶³). There is strong evidence that the incidence and severity of periodontitis is influenced in part by diabetes mellitus and the level of blood glucose level (*Nishimura, Takahashi et al, 1998*⁸⁰).

But because of the frequently mild and asymptomatic nature of diabetes in its early stages, many individuals with undiagnosed diabetes are likely to have had diabetes for several years before being diagnosed (*Harris M I, Klein R et al, 1992*⁴⁶). This points to the fact that a large number of patients with periodontitis may have undiagnosed diabetes

mellitus. By the time these individuals are diagnosed, the beta cell function may have declined substantially (*U.K. Prospective Diabetes Study Group, 1995¹¹²*) and significant damage may already have occurred. Thus, there is a critical need to increase opportunities for diabetes screening and early diabetes detection.

Periodontitis by itself is associated with gingival bleeding. Investigators have found that if the patient is also diabetic, gingival bleeding will be more severe . Bleeding from gingival tissues is found to be further pronounced if the condition is poorly controlled (*Ervasti T, Knuuttila et al, 1985²⁹*) or in association with undiagnosed diabetes (*Hage H W, Kirkham D M 1978⁴⁹*).

Sulcular bleeding is a normal or routine consequence of periodontal examination due to inflammation of the tissues regardless of whether the patient is diabetic or not (*Ervash T, Knuulitu M, 1985²⁹*). A dental clinician could use this sulcular blood to test for glucose levels, instead of perforating the patient's finger tip, using a self-monitor device, glucometer. The patient can be referred to a physician for further evaluation for diabetes when warranted. This is a safe, noninvasive and a rapid technique to assess the diabetic status during routine periodontal examinations (*Robert C Parker, Rapler et al, 1993⁹³*).

FACTORS ASSOCIATED WITH DIABETES THAT INCREASES THE SEVERITY OF PERIODONTITIS AND THEIR MECHANISMS OF ACTION.

1. **BACTERIAL PATHOGENS:-** The glucose content of gingival fluid and blood is higher in individuals with diabetes (*Ficava A I, Levin M P et al,1975³²*). Patients with type 1 diabetes mellitus and periodontitis have a subgingival flora composed of Capnocytophaga, anaerobic vibrios and an abundant Actinomyces species (*Gusberti ,*

Grossman N, Loesche W, 1982⁴¹, Mashimo P, Slots J et al, 1983⁸³). Black pigmented species, especially *P.gingivalis*, *P.intermedia* and *C.rectus* are prominent in severe periodontal lesions of Pima Indians with type 2 diabetes (*Genco RJ, Zambon JJ, 1987³⁷, Zambon JJ, Reynolds, Fisher JB et al, 1988¹¹⁴*). A greater oxygen intake and a lower glucose oxidation rate by gingival tissue from diabetics was reported which could predispose the gingival inflammation.

2. **POLYMORPHONUCLEAR LEUCOCYTE FUNCTION (PMN):-** The increased susceptibility of diabetic patients to infection has been hypothesized as being caused by PMN deficiencies resulting in a) impaired chemotaxis, b) defective phagocytosis or c) impaired adherence, diapedesis and intracellular activities in diabetics (*Mcmullen JA, vanDyke TE et al, 1981⁶⁸*). In patients with poorly controlled diabetes, the function of PMNs and monocytes / macrophages is impaired. As a result the primary defense against periodontal pathogens is diminished and bacterial proliferation is unchecked.

3. **ALTERED COLLAGEN METABOLISM:-** Metabolic irregularities induced by diabetes mellitus have been associated with abnormalities of collagen turnover. Several studies have reported an impaired metabolism of gingival and periodontal ligament fibroblasts under hyperglycemic conditions resulting in lower mitotic activity and growth as well as increased collagen activity (*Schmidt AM, 1993⁹⁶*).

In hyperglycemic state, accumulated glycogen end products (AGEs) formation is excessive. The collagen that is cross-linked by AGE formation makes it

less soluble and less likely to be normally repaired and replaced, impedes cellular migration, impairs tissue integrity as a result of damaged collagen remaining in the tissue for longer periods, collagen in poorly controlled diabetes is aged and more susceptible to breakdown (*Grossi SG, Zambon JJ, 1994*⁴⁰). Poor glycemic control with the associated increase in AGEs, renders the periodontal tissue more susceptible to destruction (*Antony M.Iacopino and Christopher W.Cutter, 2000*⁵).

RELATIONSHIP OF DIABETES MELLITUS AND PERIODONTAL DISEASE.

Improved methods of assessing metabolic control in diabetics, assessing periodontal status, microbial risk factors and risk indicators in crevicular fluid and statistical analysis have provided new information to classify the relationship between diabetes and periodontal diseases.

*Hirschfeld, 1934*⁴⁷ thoroughly investigated the influence of diabetes on the periodontium and states that although it is difficult to make definitive conclusions about the specific effects of diabetes on periodontium, a variety of changes have been described, including a tendency towards enlarged gingiva, sessile or pedunculated gingival polyps, polypoid gingival proliferations, abscess formation, gingival polyps, polypoid gingival proliferations, abscess formation, periodontitis and loosened teeth.

*Kent A Hove, et al 1970*⁵⁷ studied a group of 28 diabetic patients together with 16 non-diabetic patients to clarify the divergence of opinion regarding the status of the periodontal structures of the diabetic when compared to non diabetic patients. They concluded that periodontal disease was directly related to the accumulation of plaque and

calculus, it increases with age in both the groups and vascular changes were more frequent (71%) in the diabetics than in the non diabetics (19%).

Tellervo Ervash, et al 1985¹⁰⁷ studied the relationship between control of diabetes and gingival bleeding in 50 adult diabetics and 53 healthy controls. They concluded that those with poorly controlled diabetes suffered significantly more from gingival bleeding than those with good or moderate control.

Joseph J Zambon, et al 1988¹¹⁴ did microbiological and immunological studies of adult periodontitis in 8 patients with non-insulin dependent diabetes mellitus. Microbiologic and immunologic data from the study suggested that B..intermedius(Prevotella intermedia), C.rectus and B.gingivalis(Porphyromanus gingivalis) are important in the etiology of periodontitis in adult patients with non insulin dependent diabetes mellitus.

John Gibson, et al 1990⁵⁵ observed that the frequency of undiagnosed type 2 diabetes mellitus was twice than expected among the general population with oral manifestations like burning mouth syndromes, prolonged fungal infections, altered taste and erosive lichen planus. The diagnosis of diabetes mellitus was confirmed by measurement of fasting blood glucose levels and glucose tolerance test.

Loe H, 1993⁶⁶ states that, perhaps the most changes in uncontrolled diabetes are the reduction in defense mechanisms and the increased susceptibility to infections, leading to destructive periodontal disease. In fact, periodontal disease is considered to be the sixth complication of diabetes.

Schmidt AM, et al 1996⁹⁶ hypothesized that one mechanism underlying advanced periodontal disease in diabetes may involve oxidant stress in the gingiva induced by the effects of advanced glycation end products (AGEs), the irreversible products of non-

enzymatic glycation and oxidation of proteins and lipids which accumulate in diabetic plasma and tissue.

*Ainamo J and Ainamo A 1996*¹ reported that apart from age and smoking another important link factor for periodontitis relates to the insulin dependent and non insulin dependent form of diabetes mellitus. Poorly controlled long duration diabetes had more periodontitis and tooth loss than well controlled or non diabetics.

*Sara G Grossi and Robert J Genco 1998*⁹⁵ reported the two way relationship between periodontal disease and diabetes mellitus. The combinations of the two pathways, infections and AGE-mediated cytokine up-regulation, helps to explain the tissue destruction seen in diabetic periodontitis and how periodontal infection may complicate the severity of diabetes and the degree of metabolic control.

*Stewart JE, et al 2000*¹⁰² performed a study which was designed to explore the effect of periodontal therapy on glycemic control in patients with type 2 diabetes mellitus and concluded that periodontal therapy was associated with improved glycemic control in persons with type 2 diabetes mellitus.

*Promsudhi et al, 2005*⁸⁵ examined the effect of periodontal therapy on glycemic control in type 2 diabetic patients and their result stated that periodontal treatment significantly improved periodontal status of the patient. They concluded that uncontrolled diabetes significantly improved 3 months after mechanical periodontal therapy with adjunctive systemic antimicrobial treatment. They also observed rapidly deteriorating diabetes without periodontal treatment.

*Demmer RT, et al 2008*²³ reported the relationship between type 2 diabetes and periodontal disease. They concluded that baseline periodontal disease is an independent

predictor of incident diabetes in the nationally representative sample of National Health and Nutrition Examination Survey

EPIDEMIOLOGIC EVIDENCE:

Research has been conducted into the inter relationship between diabetes and periodontal disease since 1960's.

Early studies by *Belting et al 1964*¹¹, *Finestone et al 1967*³³, *Cohen 1970*¹⁹, *Sznajder et al 1978*¹⁰⁴, *Cianciola et al 1982*¹⁷, *Shlossman et al, 1990*⁹⁷ indicate that diabetes mellitus may influence the prevalence and severity of periodontal disease. Other studies such as by *Hove K et al 1970*⁵⁰, *Nichols C et al 1978*⁷⁸, *Barnett ML et al 1984*⁹ failed to note any relationship between diabetes mellitus and periodontal disease.

One of the largest scale studies to date was carried out in the Pima Indians of the Gila River Indian Community in Arizona who have the highest recorded prevalence of type 2 diabetes mellitus. In a cross sectional survey of the community, it was found that for all age groups, the Pima Indians with diabetes had higher prevalence of periodontal disease than those without the disease. These and other controlled studies by *Nelson et al 1990*⁷⁶, *Emrich et al 1991*²⁷, *Bridges et al 1996*¹², *Firatli et al 1997*³⁴, *Collin et al 1998*²⁰ reported at least a two-fold increase in the risk for periodontal disease in diabetes when compared with healthy controls.

*Tervonen and Knuttila 1991*¹⁰⁸ revealed that well controlled diabetes subjects had better periodontal health than the controls. In a later study, *Tervonen and Oliver 1993*¹⁰⁹

reported an increase in prevalence, severity and extent of periodontitis in patients with poor metabolic control.

Studies by *Guzman et al 2003*⁴², *Campus et al 2005*¹⁴ have reported greater prevalence and more severe periodontal disease in diabetes mellitus subjects. Poorer glycemic control as demonstrated by *Engebretson SP 2004*²⁸ was found to be associated with elevated GCF IL -1 β which may explain the association between poor glycemic control and more advanced periodontal disease.

A pilot study carried out by *Lim LP 2003*⁶⁵ on a cohort of patients with diabetes indicated that individuals with diabetes have more severe periodontal disease.

A meta-analysis by *Khader YS et al 2006*⁵⁹ compared the periodontal status of diabetics with that of non-diabetics. The author concluded that based on the average values, diabetics had poorer oral hygiene as measured by plaque index, more severe gingival disease as measured by gingival index, and higher severity of periodontal disease based on probing depth and clinical attachment level.

Several recent studies by *Gaganpreet Kaur et al 2009*³⁶, *Ting-Ting Wang et al 2009*¹¹⁰, *Chavary et al*¹⁶, *Jyotika L Fernandes et al 2009*⁵⁶ have reported that diabetes mellitus was associated with prevalence and extend of periodontal disease and tooth loss.

DIABETES – DIAGNOSTIC TESTS:

- Urine sugar test.
- Urine ketone test.
- Oral Glucose Tolerance Test (OGTT).

- Intravenous Glucose Tolerance Test (IVGTT).
- Blood Glucose Test.
 - Fasting Plasma Glucose.
 - Random Plasma Glucose.
- Glycosylated Haemoglobin (HbA1c).
- C-Peptide blood test.
- Self Monitoring Blood Glucose.

DISCOVERY OF DIAGNOSTIC TESTS.

Different methods of testing for sugar were developed such as chemical (Copper reduction), fermentative (with yeast) and by the physician (Poloroscopic).

COPPER REDUCTION METHOD:

Introduced by *Benedict in 1907*, copper reduction test is in use for a long time because of its simplicity and convenience. Clinitest is a copper reduction method of testing the urine for sugar. It is a reagent tablet that contains copper sulphate, sodium hydroxide, sodium carbonate and citric acid into a tablet. The procedure is a modified version of Benedict's qualitative test. It is rarely used in current practice. This test lacks sensitivity and specificity *Silink M. 1982* ⁹⁸.

ENZYMATIC METHODS:

The enzymatic method is based upon the beta glucose oxidase or "notatin" discovered in *1928 by Muller D* ⁷¹. This method has great specificity and do not respond to any other physiologic constituent except glucose. The ferment promoted the oxidation of glucose by molecular oxygen to gluconic acid.

PHOTOMETRIC MEASUREMENT:

In this method, special equipment is used to measure the glucose oxidation reaction in a polarographic oxygen electrode and paper strips impregnated by glucose oxidase reagents are used to measure glucose in blood, serum and urine. *Fales in 1963*³¹ evaluated and published selected glucose oxidase method.

DEXTROSTIX:

Dextrostix is a firm cellulose strip impregnated with enzyme reagents that gives approximate results for glucose concentration with one drop of whole blood (capillary or venous). The reagent area contains glucose oxidase, peroxidase and a chromogen which varies in colour and intensity depending on the amount of glucose present. Dextrostix was introduced by Ernst C Adams in 1964.

Enzymatic methods of glucose determination are classified into 3 groups:-

1. methods with glucose oxidase.
2. methods with hexokinase, and
3. methods with glucose dehydroxygenase.

1. **Glucose oxidase method:** It is based on glucose oxidation to gluconate by the action of glucose oxidase (GOD) enzyme and formation of hydrogen peroxide, which oxidizes a chromogenic substance to a coloured product under the action of peroxidase (POD). The intensity of absorbance of thus formed stained products is proportional to the concentration of glucose in the sample.

2. **Hexokinase method:** In 1965, *Stein*¹⁰¹ coupled hexokinase enzyme with glucose-6-phosphate dehydrogenase to measure glucose. *Nesse, 1982*⁷⁵ published the successful

applications of this method to the analysis of glucose in body fluids as a practical direct method. *Nesse and coworkers, 1982*⁷⁵ also proposed this method as a reference method.

3. **Glucose dehydrogenase method:** by the action of glucose dehydrogenase enzyme D-glucose and NAD are transformed to D-gluconolactone and NADH. The amount of reduced coenzyme formed is proportional to the concentration of glucose.

SELF MONITORING OF BLOOD GLUCOSE.

Progress has been dramatic in the methods to determine the concentration of glucose from the original test, followed by reagent tablets (Clinitest) or powder (Gelatest) and in the 1960s a variety of urine glucose test strips, ie test-tape / Glukotest, Clinistix, Dreyapak and Combur-test (glucose, protein and pH value). The late 1960s saw the introduction of Dextrostix and the Reflo test strips for use with reflectance meters and then the Haemo-Glukotest for visual determination. It was soon realized that the patients were capable of using such meters and their benefits outweighed the demerits thereby leading to the introduction of the concept of self-monitoring of blood glucose (SMBG).

Further developments have centered on refining the test strips, blood sampling techniques (thereby reducing the number of blood samples required), accuracy of glucose determination, and the time from sampling to results.

*Keen and Knight, 1962*⁵⁸ – introduced self-sampling for blood sugar determination. The enzymatic methods have been used extensively for home glucose testing.

In 1964, *Rennie et al*⁹⁰ described a rapid(1mm) enzyme test strip (Dextrostix, Ames, Miles Laboratories, Elkhart) – based semi-quantitative method for estimating capillary whole blood sugar.

In 1966, Tom Clemens along with Anton H Clemens invented the first blood glucose-meter. It was a reflectance meter which could read reflected light. The same dextrostix were used and the concept was that a beam of light was played on blue colour and darker the blue, the less light could be reflected. The reflected light was sent to a photoelectric cell, which in turn gave a read out, which in this case was an ammeter with a swinging needle.

In 1972, a Japanese company, Kyoto Daiichi, developed an instrument called EYETONE. Ames reflectance-meter was very expensive, heavy and bulky. In contrast, Eyetone was less expensive, more light-weighted and easier to operate. Following this out of England came a new meter called Glucocheck. It is small, compact and patient oriented meter. Also it was the first patient oriented meter.

In late 1970's, Accucheck was introduced in the market. Medisense introduced the first biosensor meter which works on electro chemical technology as against the reflectance photometry, which was the principle of earlier invented glucometer.

In 1974, the American Diabetic Association (ADA) recommended that screening for diabetes be done using plasma (or serum) glucose methods alone and that urine testing be abandoned because of the insensitive and non-specificity of clinitest methods.

Drawbacks of urine tests:-

Though urine tests are often done to determine the increase in blood glucose levels, the urine concentration of glucose depends on renal function, fluid intake and other factors in addition to blood glucose levels.

If any other reducing substance is present in urine, it could result in false-positive reactions. It doesn't provide information of the current blood glucose level, instead it shows,

what blood glucose levels were several hours ago, when the body expressed excess glucose through the kidney.

Ascorbic acid, salicylates, cephalosporin's and many other drugs have been known to interfere with the results obtained with the urine test.

Errors in Self Monitoring Blood Glucose:

The laboratory method is considered as the “gold standard” or true measure of blood glucose. The deviations seen in self monitoring system from the true values are viewed as errors. This may be due to random factors (chance variation), systemic factors (bias) or a combination of random and systemic factors. Random error increases the imprecision of the measurements. Manual timing and wiping have been recognized as significant sources of error when using self monitoring devices. Error grid analysis defines 5 major zones or categories of errors that range from zone A to zone E.

The zone indicates how adequate the therapeutic decision taken on the basis of the SMBG system result would be compared with the decision that would have been taken on the basis of the laboratory result.

Zone A: clinically accurate measurements. These measurements are within 20% of the laboratory measurements or are in the hypoglycemic range ($<3.9\text{mmol l}^{-1}$).

Zone B: measurements that deviate from the laboratory values by more than 20%, these measurements could lead to either no change in treatment or benign treatment changes.

Zone C: Measurements that would deviate from the laboratory by more than 20% and would lead to unnecessary corrective treatment errors.

Zone D: Represents “dangerous failure to detect and treat errors”. These errors occur when the patient generated measurements are within 3.9 to 10 mmol l⁻¹ range but the true glucose level are outside this target range.

Zone E: Is defined as “erroneous treatment zone”. Patient generated measurements in this zone are opposite to the reference value, and corresponding treatment decision would therefore be opposite to that called for.

It has been recommended by the Consensus Development Conference on self monitoring of blood glucose that the goal of device manufactures should be to make the future portable glucose monitors (SMBG) capable of producing results with an analytic error of $\leq 5\%$.

RECENT AND WELCOME ADVANCES INCLUDE:

- “Alternate site testing” – the use of blood drops from sites than the finger, usually the palm or forearm. This alternate site testing uses the same test strips and meter, is usually pain free and gives the finger tips a needed break if they became sore. The disadvantage of this technique is that there is usually less blood flow to alternate sites, which prevents the reading from being accurate when the blood sugar level is changing.
- “No Coding” systems. Older systems required “coding” of the strips to the meter. This carried a risk of ‘miscoding’, which can lead to inaccurate results. Two approaches have resulted systems that no longer require coding. Some systems are

“autocoded” where technology is used to code each strip to the meter. The other is a ‘single code’, thereby avoiding the risk and miscoding.

- “Multi-test” system – this system use a cartridge or a disc containing multiple test strips. Advantage is that the user doesn’t have to load individual strip each time.
- “Downloadable meters – Most new systems come with software that allows the user to download meter results to a computer.

ALTERNATIVE SITE TESTING.

Rationale for alternate site testing: Blood glucosemeters that test blood glucose level on places where fewer nerve endings are present and are much less painful than traditional finger tip meters.

Brands of alternate site glucometer:

First to be introduced was Amira medical manufacturer’s AtLast, which became available in December 1999.

The Therasence meters, of Abbott laboratories began to be marketed in 2000. Therasence free style takes the least blood sample of all the available meters.

The Medisence meters, also by Abbott Laboratories is one of the newest alternative site meter. Medisence Sof-Tact is the first fully integrated, fully automated virtually painless blood glucose monitor can be used on the forearm, upper arm or base of the thumb, in relation to the fingertip.

Sentris-100, a product of glucolight is a hospital based monitor that will make continuous, non-invasive measurement and monitoring of blood glucose a reality. It is under clinical trial that began in 2009.

LifeScan, Inc a Johnson & Johnson company manufactures One-Touch Ultrameter, gives the test result in just 5 seconds. It requires just a speck of blood. Blood from forearm can be used which is even less painful.

SITES FOR ALTERNATE BLOOD GLUCOSE MONITORING:

{ Approved by Food and Drug Administration (FDA) }

1. palm
2. forearm
3. upper arm
4. thigh
5. calf muscle
6. abdomen
7. ear lobe (not convenient)
8. foot (not recommended)
9. gingiva.

GENERATIONS OF GLUCOMETER

First Generation Meters:

First generation glucometers have a chemical (glucose oxidase) on the test-strip that when exposed to glucose from blood changes colour. The darker the colour the more the

glucose is present. The colour variation is measured photometrically or colorimetrically to give a glucose reading.

Second Generation Meters:

Second generation glucometers have reagents (glucose oxidase and potassium ferric cyanide) on the test strip. When these are exposed to blood containing glucose, the reaction causes electrons to increase their electrical activity. The more glucose present the more electrical activity is generated. These second generation glucometers can further be subdivided according to the electrochemical principle used.

(1). Amperometry.

(2). Colorimetry.

Third Generation Glucometers:

These meters test blood glucose levels continuously and automatically. They are also being developed that will allow glucose testing without an actual body fluid being used. Promising technologies include infrared and radiation.

Stein GM et al 1969¹⁰⁰, keeping the idea of early detection of diabetes in dental patients performed a survey of periodontal population with a paper reagent strip utilizing gingival blood for detection of asymptomatic, undiagnosed diabetes mellitus in dental patients. Dextrostix was a convenient, quick and inexpensive method for screening of blood glucose. Their data suggested that dextrostix reagent strip could be useful in diabetes screening surveys.

Antony J Ficara, Marvin P Levin, Marvin F Grower, 1975 ⁴ compared the glucose and protein content of gingival fluid from diabetes and non-diabetics. The glucose content of both the gingival fluid and blood of the diabetics was significantly elevated above those seen in the control group. The glucose content of the gingival fluid from the diabetics also showed a significant correlation of the blood glucose levels found in non-diabetic patient.

Tellervo Ervasti et al 1985 ¹⁰⁷, found no difference between the diabetic group and the control group in either the amount of etiologic factor or in the degree of gingival changes. But, they found significantly more gingival bleeding in poorly controlled diabetic than in good or moderately controlled diabetic subjects.

Peggy Tsuitsui et al, 1985 ⁸² compared intraoral blood to finger prick blood samples to determine if it could be used as a suitable alternative. During routine oral prophylaxis, bleeding was elicited from periodontal sulci. Blood was also obtained from finger puncture. The study provides strong correlation for the feasibility of using periodontal sulcular blood as an alternative to blood obtained from a finger puncture for glucose measurement.

According to *Tsuitsui P, Rich SK* ¹¹¹ use of intraoral sampling would require,

1. selection of an inflamed gingival site.
2. adequate blood flow to cover the reagent strip.
3. care to avoid reagent strip abrasion on restorations and tooth surfaces.
4. expeditious application of blood to reagent strip.
5. observance of reagent strip shelf life, handling and calibrations of measuring device according to manufacturers instruction.

Rosenthal IM et al 1988 ⁹⁴ evaluated and supported that there was a higher gingival index score and sulcular bleeding index score in insulin dependent diabetes mellitus patients with neurological complications than those without complications.

Robert C Parker et al 1993 ⁹³ collected gingival crevicular blood samples and finger puncture capillary blood samples and tested for blood glucose levels by self monitoring method and venous blood was collected for measurement in a laboratory glucose analyzer. Their results suggested that gingival crevicular blood collected by a small plastic pipette and measured in a glucose self monitoring unit was feasible for in-office blood glucose testing with measurements comparable to those obtained using the finger-puncture method.

Beikler T et al 2002 ¹⁰ conducted a study to evaluate whether blood oozing from gingival tissues during routine periodontal examination could be used for determining glucose levels. Their result suggested that blood oozing during routine periodontal examination may be used for diabetes mellitus screening in a dental office setting.

Nicer T, Lopatynshi J, 2003 ⁷⁷ reported a study by decentralized health care system based on family doctors carrying out a comparative study on determining 131 patients glucose concentration level in capillary blood, whole venous blood (using Roches Glucotrend Glucometer) and venous blood serum by means of the laboratory method.

Muller HP, Behbehani, 2004 ⁷² tested 46 patients and measurement in gingival crevicular blood were compared with those of conventional capillary finger stick blood using sensitive self monitoring device. They reported that the agreement between the two measurements to be low and hence failed to provide any evidence for the usefulness of GCB for testing blood glucose using routine periodontal examination.

Hamid MH, Chishti AL, Maqbool S 2004 ⁴⁴ in their study conducted at neonatal unit of children's hospital, Lahore. All neonates were screened for hypoglycemia using capillary blood on Accutrend alpha glucometer. All these neonates presented with known factors or suggestive clinical features. Simultaneously venous blood glucose values were estimated using Hitachi 902 auto-analyzer by hexokinase method. Hypoglycemia was detected in 112 samples (38.4%) of the 292 paired samples studied. Correlations of blood glucose levels with both glucometer values and laboratory values were excellent throughout the range.

Ho HT, Yeung W K, Young BW ⁴⁸ studied the performance of five available glucometers for the detection of low blood glucose in new born infants.

Muller HP, Behbehani E, 2005 ⁷³ in their correlation analysis revealed that measurements of glucose levels in capillary finger blood from left and right finger tips were highly correlated pointing to excellent performance of the device, where as capillary finger blood and gingival crevicular blood measurements were moderately but highly significantly correlated.

Khader YS, Judeth A et al, 2006 ⁵⁹ concluded in their study among Jordanian patients that gingival crevicular blood glucose can provide an acceptable sources of measuring blood glucose level. The technique to obtain an acceptable blood sample from gingival crevices is not always feasible which would limit its application in clinical practice.

Ardakani MR, Amir Moeintaghavi, Haerian 2009 ⁶ studied 30 diabetic and 30 non-diabetic patients with periodontitis to routine periodontal examination. The blood collected from gingival sulci was tested using a test strip of glucose self-monitoring device. The results of this study suggested sulcular blood from a routine periodontal examination may be used for diabetes mellitus screening.

Strauss SM, Alla j Wheeler et al, 2009 ¹⁰³ examined conditions under which gingival crevicular blood could be used to obtain a useful glucose reading to screen for undiagnosed diabetes for routine dental visits. They concluded that G.C.B samples were suitable to screen for diabetes in persons with sufficient bleeding on probing (BOP) to obtain a sample without touching the tooth or gingival margin.

Ramona Elena, Silvia Marti 2010 ⁸⁷ evaluated the correlation established between the value of glicemy from Capillary Blood Glucose Levels (CBGL) and Sulcular Blood Glucose Level (SBGL) respectively and they concluded that a family history of diabetes, as well as a more severe periodontal disease are found in diabetics. Having in view that 50% of persons suffering from diabetes are not diagnosed as such, an assessment of their glicemy from gingival sulcular blood during routine stomatological examinations may be recommended as an adequate method for the identification of potential diabetics due to the correlation with the glicemy from the capillary blood.

MATERIALS AND METHOD

STUDY SUBJECTS:

Sixty subjects were selected for this study from the out-patient department, Department of Periodontics, Tamilnadu Government Dental College and Hospital, Chennai-600 003.

30 subjects were known diabetes mellitus patients and they were categorized as Group I and the other 30 subjects were non-diabetic subjects with untreated moderate to severe chronic periodontitis categorized as Group II.

SOURCE:

Out-patient Department,
Department of Periodontics,
Tamilnadu Government Dental College and Hospital,
Chennai – 600003.

INCLUSION CRITERIA (Both groups):

1. Age group: 40 – 60 years.
2. Both males and females.
3. Patients with moderate to severe chronic periodontitis (*Flemming TF, 1999*³⁵).

EXCLUSION CRITERIA (Both groups):

1. Patients with anemia/ haematological disorders (*Nicola O’Connell, 2003*⁷⁹).

2. Patients under anticoagulant therapy.
3. Patients with systemic disorders such as cardiovascular, hepatic , immunologic, renal disorders or other organ failures.
4. Patients requiring antibiotic premedication.
5. Patients with a history of periodontal treatment during the past 6 months.
6. Areas of periodontium with suppuration.

DESIGN OF THE STUDY:

A cross-sectional study was performed on a total of 60 subjects (26 males and 34 females).

The subjects were divided into two groups as follows:

GROUP I

Thirty diabetic subjects with chronic periodontitis, diagnosed clinically with clinical attachment loss (CAL) of $\geq 3\text{mm}$ and with radiographic evidence of bone loss .

GROUP II

Thirty non-diabetic patients with chronic periodontitis, diagnosed clinically with clinical attachment loss (CAL) of $\geq 3\text{mm}$ and with radiographic evidence of bone loss.

STUDY PROTOCOL:

1. Institutional ethical committee approval.
2. Medical history and informed consent – obtained.
3. Intraoral examination was done using a mouth mirror and a Williams periodontal probe under artificial light.

4. Periodontal examination was done – Clinical parameters namely Gingival bleeding index, Plaque index, Pocket probing depth and Clinical attachment level were recorded.
5. Orthopantomogram (OPG) was taken to inspect the radiographic parameters and the periodontal status.
6. Maxillary anterior sextant was chosen as the site for blood sampling as they offer ideal access for collecting gingival crevicular blood.
7. The random blood glucose levels were analyzed from both gingival crevicular blood and finger prick blood using glucose self monitoring device.
8. Statistical analysis was done.

CLINICAL PARAMETERS:

The clinical parameters recorded for all the selected subjects:-

1. Gingival bleeding index – *Ainamo and Bay 1975*².
2. Plaque index – *Silness and Loe 1964*⁹⁹
3. Probing pocket depth in mm (PPD) – *Carranza 10th ed*¹⁵.
4. Clinical attachment level in mm (CAL) – *Carranza 10th ed*¹⁵.

GINGIVAL BLEEDING INDEX (*Ainamo and Bay, 1975*²)

Teeth examined : All teeth.

Surfaces examined : 4 sites for each tooth (mesial, distal, buccal, lingual).

The presence or absence of bleeding is determined by gentle probing of the gingival crevice with a periodontal probe.

Criteria for scoring

Positive score (+) : Presence of bleeding within 10 sec.

Negative score (-) : Absence of bleeding.

$$\text{Percentage of bleeding sites} = \frac{\text{Total number of positive score}}{\text{Total number of surface of all teeth}} \times 100$$

PLAQUE INDEX (*Silness and Loe, 1964*⁹⁹)

Teeth examined : All teeth.

Surfaces examined : Distofacial, Facial, Mesiofacial, Lingual.

Criteria for scoring:

Score 0 : No plaque.

Score 1 : A film of plaque adhering to the free gingival margin recognized by running a probe.

Score 2 : Moderate accumulation that can be seen by eye.

Score 3 : Abundance of soft deposits.

$$\text{Plaque index per tooth} = \frac{\text{Total score}}{4}$$

$$\text{Plaque index per individual} = \frac{\text{Total P I per tooth}}{\text{Total number of teeth examined}}$$

Interpretation:

0	- Excellent.
0.1 – 0.9	- Good
1.0 – 1.9	- Fair
2.0 – 3.0	- Poor

PROBING POCKET DEPTH (in mm) (PPD) (*Carranza 10th ed*¹⁵)

It is the distance between the gingival margin and the base of the pocket. It was measured by inserting a probe into the pocket. In this study the depth of the pocket was measured using a Williams periodontal probe.

PROBING TECHNIQUE:

The probe was inserted parallel to the vertical axis of the tooth and ‘walked’ circumferentially around each surface of each tooth to detect the areas of deepest penetration. The entire circumferences of the pocket should be inspected so as not to miss any narrow pocket entrance. As the resistance to future penetration was noted, readings were recorded to the nearest millimeter. Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, Distolingual).

CLINICAL ATTACHMENT LEVEL (in mm) (CAL) (*Carranza 10th ed*¹⁵)

Clinical attachment level is the distance between the base of the pocket and a fixed point on the crown, such as the cementoenamel junction (CEJ) to the base of the pocket.

Changes in the level of attachment can be caused only by gain or loss of attachment and thus provide a better indication of the degree of periodontal destruction.

When gingival margin is located on the anatomic crown, the level of attachment is determined by subtracting from the depth of the pocket, the distance from the gingival margin to the cementoenamel junction. If both are the same, the loss of attachment is zero. When gingival margin coincides with the cementoenamel junction, the loss of attachment equals the pocket depth. When gingival margin is located apical to cementoenamel junction, the loss of attachment is greater than the pocket depth and therefore the distance between the cementoenamel junction and the gingival margin should be added to the pocket depth.

ARMAMENTARIUM

CLINICAL EXAMINATION:

1. Disposable face mask.
2. Surgical gloves.
3. Mouth mirror.
4. Williams periodontal probe .
5. Tweezer.
6. Kidney tray.
7. Sterile cotton.

SAMPLE COLLECTION AND BLOOD SUGAR LEVEL ESTIMATION:

1. Sterile cotton.
2. Surgical spirit.
3. Blood Glucometer (Accu-Chek Active).
4. Test strips (Accu-Chek Active).
5. Micropipette.
6. Sterile lancets.
7. Williams periodontal probe .

GINGIVAL CREVICULAR BLOOD:

SITE SELECTION:

Each patient was examined intra-orally for the visual signs of gingival inflammation and sites with bleeding on probing were selected amongst maxillary anterior teeth. Samples of gingival crevicular blood were obtained at random from diabetic and non-diabetic patients with chronic periodontitis.

SAMPLE COLLECTION:

Maxillary anterior teeth with $CAL \geq 3mm$, were chosen as the sample collection site to establish the glucose level as they offer ideal access for obtaining gingival crevicular blood. For each measurement only one site with bleeding on probing was selected. Sites with suppuration were excluded from the study. The area was isolated with cotton rolls to prevent saliva contamination and air dried. To obtain a clean sample, probing was repeated, when necessary, until a sufficient quantity of blood (~ 2 to $3 \mu l$) was present to gather a sample. Every attempt was made to obtain the blood sample on the strip by a clean catch without contacting either the tooth surface or the periodontal tissues.

After selecting the sampling site, a Williams periodontal probe was used to probe along the periodontal pocket. As soon as the probe was removed, the gingival crevice was observed for bleeding. The blood that got collected first at the gingival sulcus was washed away, in an attempt to minimize the contamination. The sampling site was isolated with gauze or cotton rolls and gently air dried. A periodontal probe was used, to probe along the periodontal pocket and observed for bleeding. At this stage, about 2 to $3\mu l$ of blood oozing from the gingival sulcus was collected with a micropipette and transferred to the test end of

the strip mounted on the glucose monitoring device. The test strip was left in place until the instrument beeped giving the blood glucose measurement in mg/dl. The reading was recorded in the proforma.

FINGER-PRICK CAPILLARY BLOOD:

SITE SELECTION:

Samples for finger-capillary blood were taken preferably from the pad of the index finger of the patient's non-dominant hand.

SAMPLE COLLECTION:

The pad of the index finger was wiped with surgical spirit and the spirit was allowed to evaporate. The finger was punctured with a sterile lancet and a drop of blood was allowed to form on the finger. The first drop of blood was discarded and as soon as the second drop of blood was formed, the test end of the strip was touched to the bleeding site and was held until the instrument gave a beep displaying the blood glucose measurement on the screen in mg/dl.

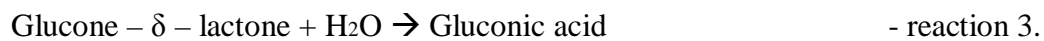
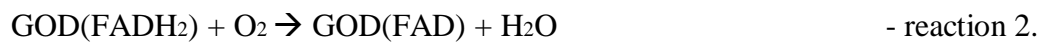
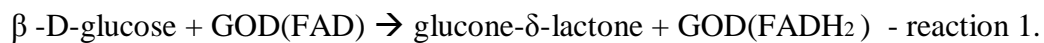
SELF MONITORING GLUCOSE METER:

A self monitoring glucometer is an electrochemical biosensor, intended for use in the quantitative measurement of the whole blood.

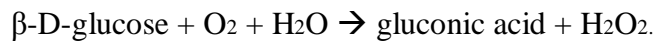
PRINCIPLE:

Glucose biosensors are generally based on the enzyme glucose oxidase (GOD). Glucose oxidase is hyper specific for β -D-glucose and any glucose present in the " α " form must be converted to " β " form before reacting. This enzyme catalyses the reaction of β -D-glucose by

molecular oxygen producing gluconolactone and hydrogenperoxide (*Wiebel MK, Bright HJ, 1971*). It is a two stage enzyme process. The process consists of enzymatic oxidation of glucose by the enzyme in which the cofactor Flavin Adenine Dinucleotide (FAD) is reduced to FADH₂ (reaction 1) followed by oxidation of the enzyme cofactor (regeneration of the biocatalyst) by molecular oxygen with formation of hydrogen peroxide (reaction 2).



The gluconolactone produced during the reaction (1) is hydrolysed (reaction 3) in aqueous media to gluconic acid (reaction 3) so that overall reaction is



Electrochemical biosensors are constructed on amperometric principle base on the oxidation or reduction of electrochemically active substances involved or produced in reaction 1 and 3 (*Turner APF, Harube I, 1987*).

The main method for the construction of electrochemical glucose biosensors involves the use of enzyme electrodes which the biologic component (enzyme) is incorporated as a part of the transducer design.

The glucose in the blood sample reacts with the glucose dehydrogenase enzyme to yield gluconolactone and produce a small electric current. This current is measured by the meter and is displayed as glucose level.

METHOD:

When the user inserts a test strip the meter burns on and displays a symbol for blood drop and battery level. The edge of the test strip is touched to the blood drop (obtained by prick) and the sample chamber on the strip fills by capillary action in approximately 2 seconds. The blood sample volume required is only approximately 2 μ l. The meter sounds a tone (beep) to let the user know that the sample chamber is full and the glucose oxidase reaction has begun. The samples were collected between 8 to 10am to assess the random blood glucose level

When the test is complete, the meter displays the glucose reading on the liquid crystal display (LCD) screen in approximately 5 seconds.

The data is collected and analyzed statistically.

LIMITATIONS :

1. Uneven layer of blood on the strip may lead to a variation in the reading as it might possibly lead to inadequate distribution of blood.
2. As adequate blood flow may not be obtained from non-inflamed gingiva, hence, it is necessary to select patients who with inflamed tissue.
3. Possible discrepancy may occur due to dilution of blood, by gingival crevicular fluid, oozing from sulcus after probing.

STATISTICAL ANALYSIS

The statistical analysis was done using the computer software program SPSS version 15 (Statistical Package for Social Science, Version 15).

Descriptive data are presented as mean \pm SD and range values.

The comparison of mean values between the two groups was calculated and Mann-Whitney U-Test was used to calculate p-value.

The comparison of mean values between the two methods in both the groups was calculated using Wilcoxon Signed Ranks test to calculate the p-value.

Spearman's rank correlation coefficient test was used to analyze the correlation between gingival and capillary methods.

STATISTICAL FORMULA'S USED FOR DATA ANALYSIS.

SPEARMAN'S RANK CORRELATION COEFFICIENT TEST:

The formula used is

$$\rho = 1 - \frac{6 \sum d_i^2}{n(n^2 - 1)}$$

where:

$d_i = x_i - y_i$ = the difference between the ranks of corresponding values X_i and Y_i , and

n = the number of values in each data set (same for both sets).

Wilcoxon Signed Rank Test

The formula is

$$Z = \left[T - \frac{n(n+1)}{4} \right] / \sqrt{\left[\frac{n(n+1)(2n+1)}{24} \right]}$$

Where n is the number of pairs. T is the sum of the ranks for the positive differences.

We compare Z to the tabulated Normal distribution.

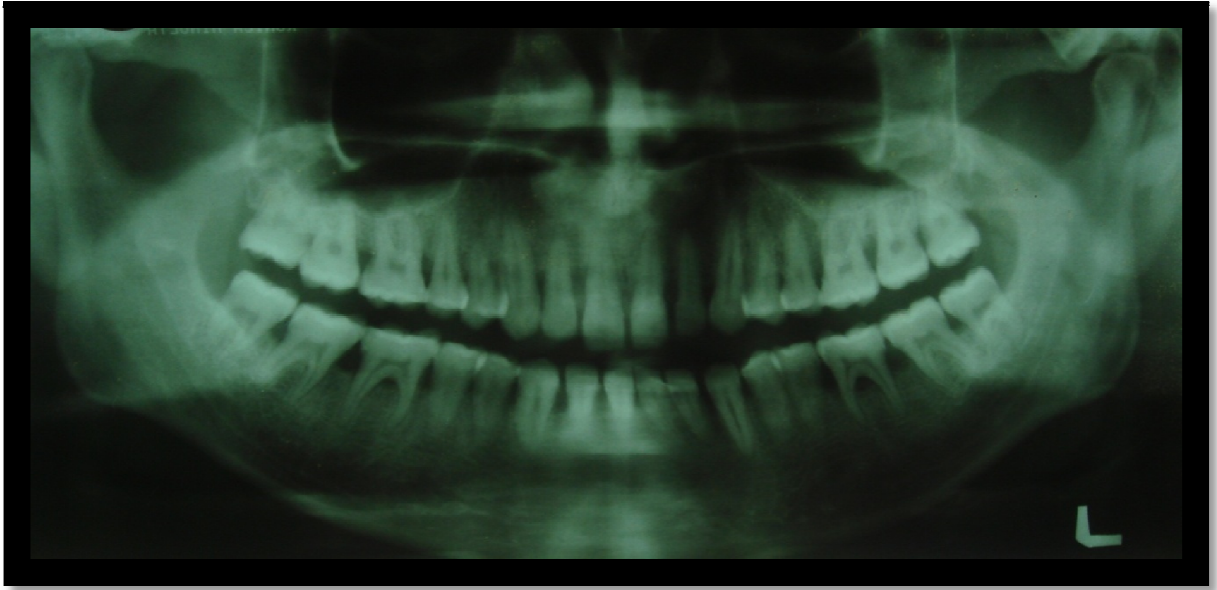
Mann-Whitney U-Test

The formula is

$$Z = \left[T - \frac{n_1(n_1 + n_2 + 1)}{2} \right] / \sqrt{n_1 n_2 (n_1 + n_2 + 1) / 12}$$

Where n1 and n2 are the sample sizes in Group I and Group II respectively.

T is the sum of the ranks for the n1 observations.



Photograph no 1: ORTHOPANTOMOGRAM



Photograph no 2: CHRONIC PERIODONTITIS



Photograph no 3: CLINICAL ATTACHMENT LOSS OF 5mm



Photograph no 4: ARMAMENTARIUM FOR CLINICAL EXAMINATION



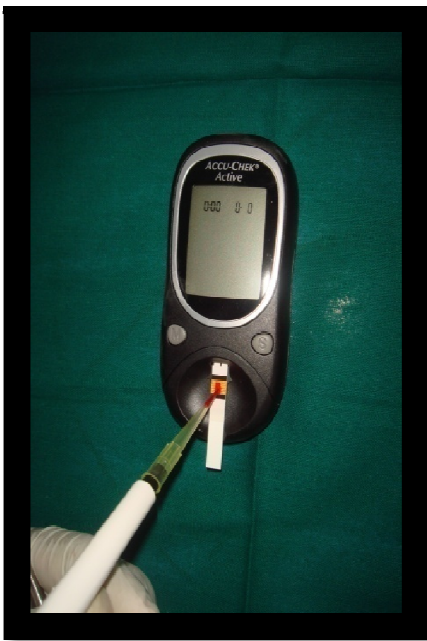
Photograph no 5: MICROPIPETETTE



Photograph no 6: GLUCOMETER, LANCET, TEST-STRIPS



Photograph no:7: MICROPIPETTE USED TO COLLECT SULCULAR BLOOD.



Photograph no 8: SULCULAR BLOOD BEING TRANSFERRED TO THE TEST-STRIP AND THE BLOOD GLUCOSE READING ON THE GLUCOMETER MONITOR



Photograph no 9: SWABING THE PAD OF THE INDEX FINGER WITH SURGICAL SPIRIT



Photograph no 10: THE LANCET IN POSITION ON THE INDEX FINGER.



Photograph no 11: A DROP OF BLOOD ON THE PAD OF THE INDEX FINGER



Photograph no 12: GLUCOMETER SHOWING READING

RESULTS

In the present study, 60 subjects were included – 30 type 2 diabetes mellitus patients with chronic periodontitis were categorized as group I and 30 non-diabetes patients with chronic periodontitis were categorized as group II.

Group I (type 2 diabetes mellitus patients with chronic periodontitis) (Table1)

This group consisted of 30 subjects (18 males and 12 females) within the age group of 40 to 60 years.

Group II : (non-diabetic patients with chronic periodontitis) (Table 2)

This group consisted of 30 subjects (8 males and 22 females) within the age group of 40 to 60 years.

From each patient of the two groups, blood samples were taken for blood glucose measurement from both the gingival crevicular blood and finger prick capillary blood. The blood glucose measurements of all the patients belonging to both the groups are shown in **Table 1 and Table 2**. Also the percentage deviation between the gingival sulcular blood glucose level and finger prick capillary blood glucose level of both the groups are shown in **Table 1 and Table 2** respectively.

The mean blood glucose values of capillary blood glucose and gingival sulcular blood glucose of both the groups are shown in **table 3** and its represented by bar diagram in **figure 1**

The **range of the gingival crevicular blood glucose measurements of the group I** patients varied from 119 to 347 mg/dl, with a mean value of 226 mg/dl and a standard deviation of 60 mg/dl.

The range of the finger-prick capillary blood glucose measurements of the group I patients varied from 130 to 366 mg/dl, with a mean value of 234 mg/dl and a standard deviation of 61 mg/dl.

The difference between the mean values of gingival crevicular blood glucose level and finger-prick capillary blood glucose level from the patients of group I is 8mg/dl and the standard deviation is 10mg/dl. (**Table 3**)

The **range of the gingival crevicular blood glucose measurements of the group II** patients varied from 85 to 496 mg/dl, with a mean value of 148 mg/dl and a standard deviation of 87 mg/dl.

The range of finger-prick capillary blood glucose measurement of group II patients varied between 91 and 525mg/dl, with a mean value of 156mg/dl and a standard deviation of 91mg/dl.

The difference between the mean value of gingival crevicular blood glucose level and finger-prick capillary blood glucose level from patients of group II is 7mg/dl and the standard deviation is 6mg/dl (**Table 3**).

The mean values of gingival crevicular blood glucose of the group I (226 ± 60) is significantly higher than in group II (148 ± 87) ($p < 0.0001$) similarly the mean value of

capillary finger-prick blood glucose level in group I (234 ± 61) is significantly higher than in group II (156 ± 91) ($p < 0.0001$).

Mann-Whitney U-Test was used to calculate the p-value. However there is no significant difference in the mean difference between the two procedures of glucose level estimation in group 1 and group II ($p = 0.11$), as seen in **table 3**.

Table 4 and figure 2 shows the comparison of the **mean values between the 2 methods** in the group I, the mean value of gingival crevicular blood glucose measurement is 226 ± 60 whereas the mean value of finger-prick capillary blood glucose measurement is 234 ± 61 . There is a mean difference of 8 ± 10 , which is statistically significant ($p = 0.001$).

Table 5 and figure 3 shows the comparison of the mean values between the two methods in group II. The mean value of gingival crevicular blood glucose method is 148 ± 87 whereas the mean value of finger-prick capillary blood glucose method is 156 ± 91 . There is a mean difference of 7 ± 8 , which is statistically significant ($p < 0.001$).

Wilcoxon signed ranks test was used to calculate the p-value.

Table 6 shows the **correlation analysis** between gingival crevicular blood glucose and finger-prick capillary blood glucose method in both the groups.

In group I, the correlation coefficient between both the methods was 0.981 which was calculated using Spearmans rank correlation coefficient, the p-value is < 0.0001 , which is significantly correlated.

In group II, the correlation coefficient between both the method was 0.976 which was calculated using Spearman's rank correlation coefficient. The p-value is <0.0001 and is significantly correlated.

In both the groups, the blood glucose value obtained using both the methods are highly positively significantly correlated.

Table 7 shows the **reliability analysis** of both the groups. In group I, the intra-class correlation coefficient between both the methods (GCBG & CBG) was 0.987 and the p-value was <0.0001 which was significantly correlated.

In group II, the intra-class correlation coefficient between both the methods (GCBG & CBG) was 0.998 and the p-value was < 0.0001 which was significantly correlated.

In both study groups, the reliability of the 2 procedure is significantly high ($p < 0.0001$).

Table 1 - MASTER CHART

GROUP I : TYPE 2 DIABETIC SUBJECTS WITH CHRONIC PERIODONTITIS

Sl. No	Age	Sex	Gingival Crevicular Blood Glucose level (mg/dl)	Finger-prick Capillary blood Glucose (mg/dl)	Percent deviation
1	42	F	215	231	6.92
2	60	M	306	313	2.27
3	49	F	246	255	3.52
4	40	F	179	168	-6.50
5	54	M	245	241	-1.65
6	43	F	226	238	5.88
7	45	M	124	130	4.61
8	48	F	215	228	5.7
9	56	M	347	366	5.19
10	46	M	119	132	9.84
11	58	M	242	255	5.00
12	60	M	195	207	5.7
13	45	M	257	263	2.28
14	42	M	149	155	3.87
15	50	F	231	247	6.4
16	53	M	257	261	1.53
17	40	F	340	326	-4.23
18	51	F	165	188	12.23
19	58	M	230	241	3.73
20	52	M	194	204	4.90
21	60	M	236	243	2.88
22	43	M	186	195	4.61
23	57	M	307	319	3.76
24	56	F	278	291	4.46
25	51	M	242	222	-9.00
26	60	M	187	190	1.57
27	41	M	154	171	9.94
28	58	F	313	330	5.15
29	56	F	247	259	4.63
30	52	F	141	139	-1.14

Table 2 – MASTER CHART

GROUP II : NON-DIABETIC SUBJECTS WITH CHRONIC PERIODONTITIS

SL.No	Age	Sex	Gingival Crevicular Blood Glucose level (mg/dl)	Finger-prick Capillary blood Glucose (mg/dl)	Percent deviation
1	57	F	97	105	8.24
2	46	F	85	91	6.59
3	49	M	96	99	3.03
4	49	F	87	97	10.3
5	48	F	90	102	11.76
6	40	F	117	124	5.64
7	56	F	113	122	7.37
8	43	F	177	188	5.85
9	51	F	229	240	4.58
10	42	F	96	94	-2.12
11	48	F	101	108	6.48
12	56	M	188	194	3.09
13	60	M	212	220	3.63
14	57	F	158	176	10.22
15	54	M	101	104	2.88
16	40	F	103	108	4.62
17	55	M	100	109	8.25
18	58	F	105	103	-1.94
19	40	F	111	116	4.31
20	58	F	111	120	7.5
21	60	F	93	97	4.12
22	53	F	113	119	5.04
23	47	F	496	525	5.52
24	43	M	121	121	0
25	44	M	101	103	1.94
26	57	F	251	267	5.99
27	40	F	291	297	2.02
28	48	M	247	255	5.16
29	53	F	132	138	4.31
30	43	F	130	133	2.25

Table 3

COMPARISON OF MEAN VALUES BETWEEN TWO GROUPS

Variable	Group I	Group II	P-value^{\$}
	Mean \pm S.D.	Mean \pm S.D.	
GINGIVAL CREVICULAR BLOOD GLUCOSE LEVEL (mg / dl)	226 \pm 60	148 \pm 87	<0.0001 (Sig.)
CAPILLARY BLOOD GLUCOSE LEVEL (mg / dl)	234 \pm 61	156 \pm 91	<0.0001 (Sig.)
Difference	8 \pm 10	7 \pm 6	0.11 (N.S.)

^{\$} Mann-Whitney U - test was used to calculate the p-value.

INFERENCE

- The mean value by gingival method in group I (226 \pm 60) is significantly higher than group II (148 \pm 87) (P<0.0001).
- The mean value by capillary method in group I (234 \pm 61) is significantly higher than group II (156 \pm 91) (P<0.0001).
- However, there is no significant difference in the mean difference between two procedures of glucose level estimations in group I and group II (P=0.11).

Table 4

COMPARISON OF MEAN VALUES BETWEEN TWO METHODS IN GROUP I

Method	Mean \pm S.D.	Difference	P-value ^{\$}
		Mean \pm S.D.	
GINGIVAL CREVICULAR BLOOD GLUCOSE LEVEL (mg / dl)	226 \pm 60	8 \pm 10	0.001 (Sig.)
CAPILLARY BLOOD GLUCOSE LEVEL (mg / dl)	234 \pm 61		

^{\$} Wilcoxon Signed Ranks test was used to calculate the p-value.

INFERENCE

- In Group I, the mean value by gingival method is 226 \pm 60 and the mean value by capillary method is 234 \pm 61. Thus, there is a mean difference of 8 \pm 10, which is statistically significant (P=0.001).

Table 5

COMPARISON OF MEAN VALUES BETWEEN TWO METHODS IN GROUP II

Method	Mean \pm S.D.	Difference	P-value ^{\$}
		Mean \pm S.D.	
GINGIVAL CREVICULAR BLOOD GLUCOSE LEVEL (mg / dl)	148 \pm 87	7 \pm 6	<0.0001 (Sig.)
CAPILLARY BLOOD GLUCOSE LEVEL (mg / dl)	156 \pm 91		

^{\$} Wilcoxon Signed Ranks test was used to calculate the p-value.

INFERENCE

- In Group II, the mean value by gingival method is 148 \pm 87 and the mean value by capillary method is 156 \pm 91. Thus, there is a mean difference of 7 \pm 6, which is statistically significant (P<0.0001).

Table 6

**RESULTS OF CORRELATION ANALYSIS BETWEEN GINGIVAL AND
CAPILLARY METHODS IN BOTH GROUP I AND GROUP II.**

Group	Correlation Coefficient*	P-Value
I	0.981	<0.0001 (Sig.)
II	0.976	<0.0001 (Sig.)

***Spearman's rank correlation coefficient**

INFERENCE

- **In both study groups, the glucose values by two methods are highly positively significantly correlated (P<0.0001).**

Table 7

RESULTS OF RELIABILITY ANALYSIS IN BOTH GROUP I AND GROUP II.

Group	Intraclass Coefficient Correlation	P-Value
I	0.987	<0.0001 (Sig.)
II	0.998	<0.0001 (Sig.)

INFERENCE

In both study groups, the reliability of the two procedures is significantly high (P<0.0001).

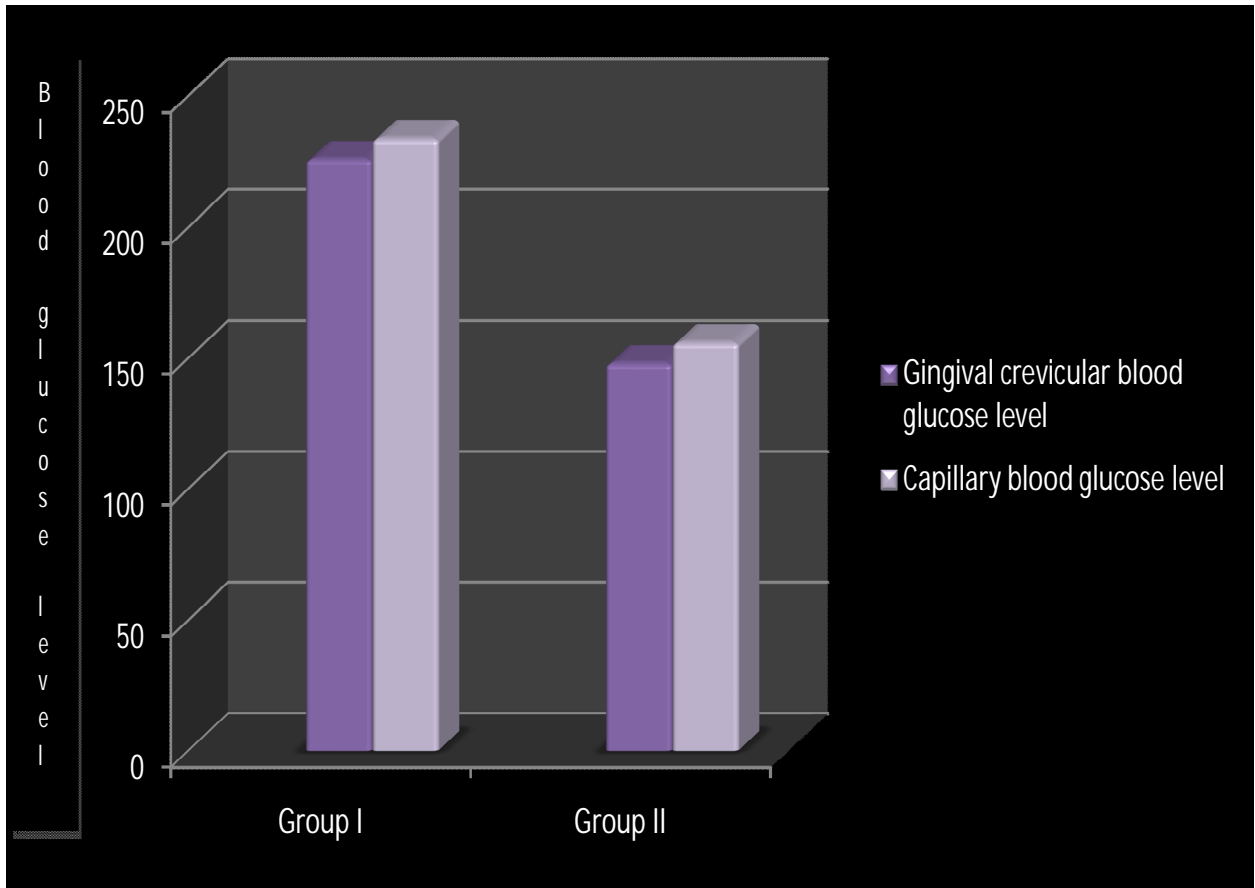


Figure 1

MEAN VALUES OF FINGER-PRICK CAPILLARY BLOOD GLUCOSE LEVEL AND GINGIVAL SULCULAR BLOOD GLUCOSE LEVEL IN DIABETIC AND NON-DIABETIC GROUPS.

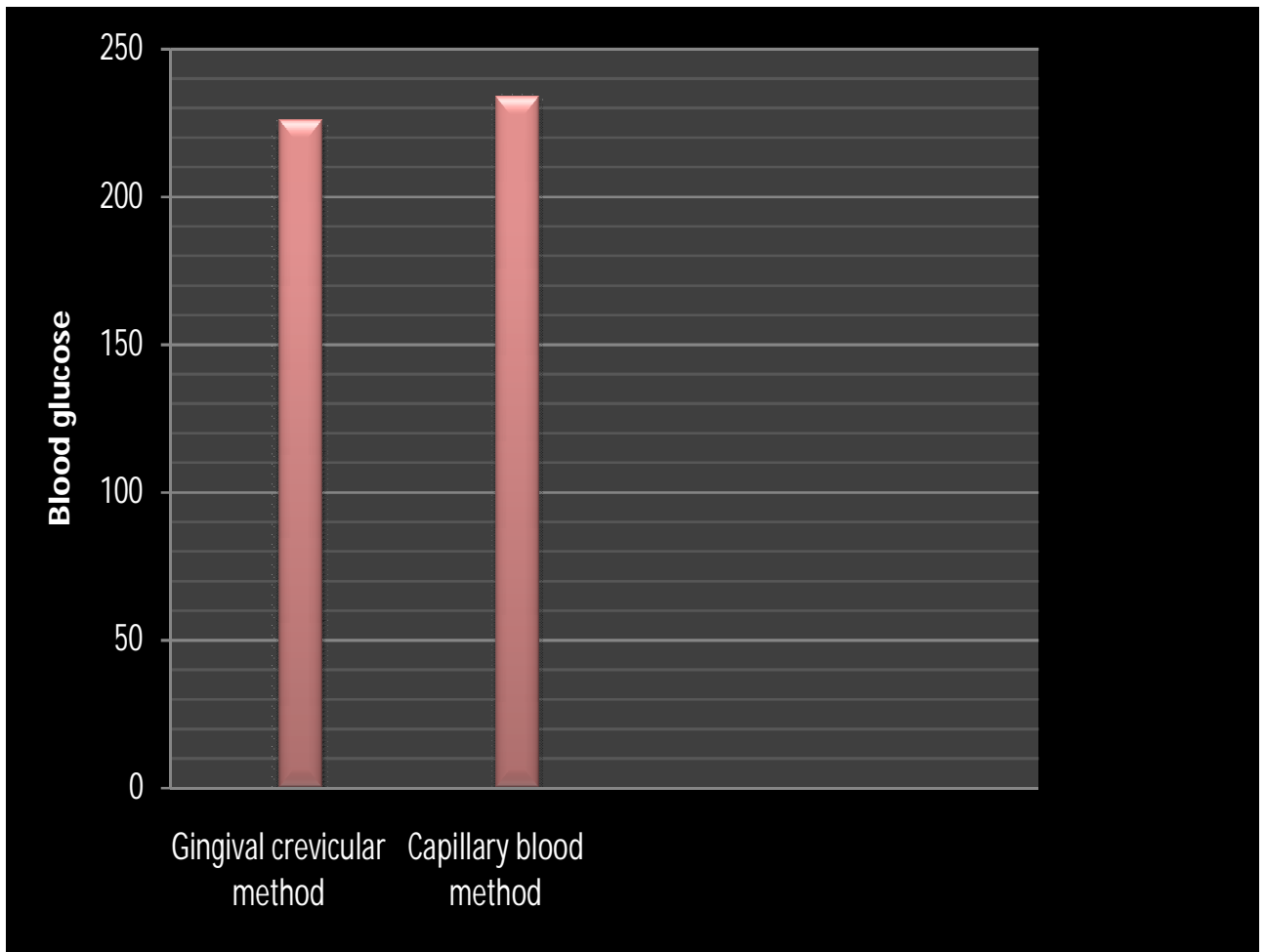


Figure 2

COMPARISON OF MEAN VALUES BETWEEN 2 METHODS IN GROUP 1

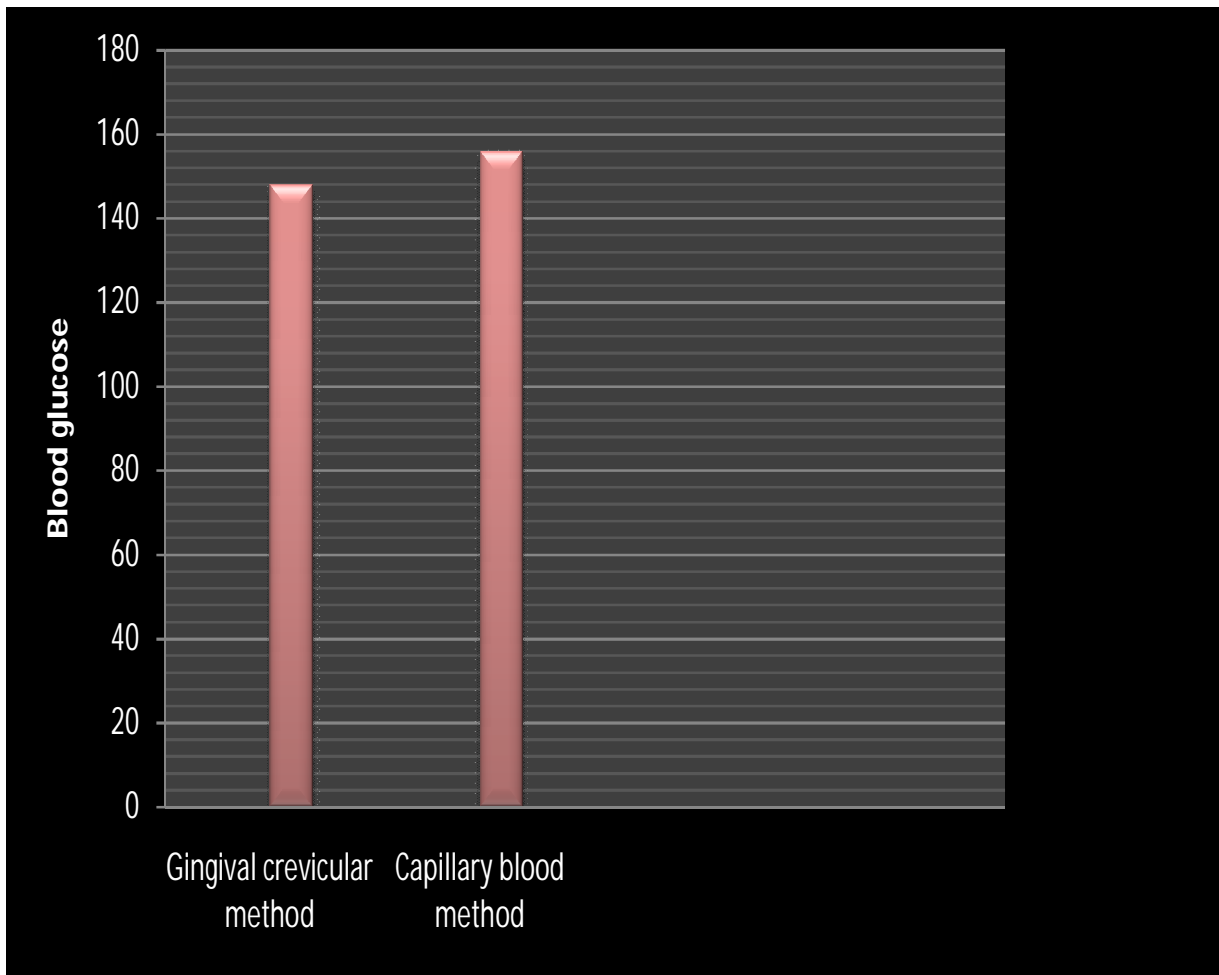


Figure 3

COMPARISON OF MEAN VALUES BETWEEN THE 2 METHODS IN GROUP II

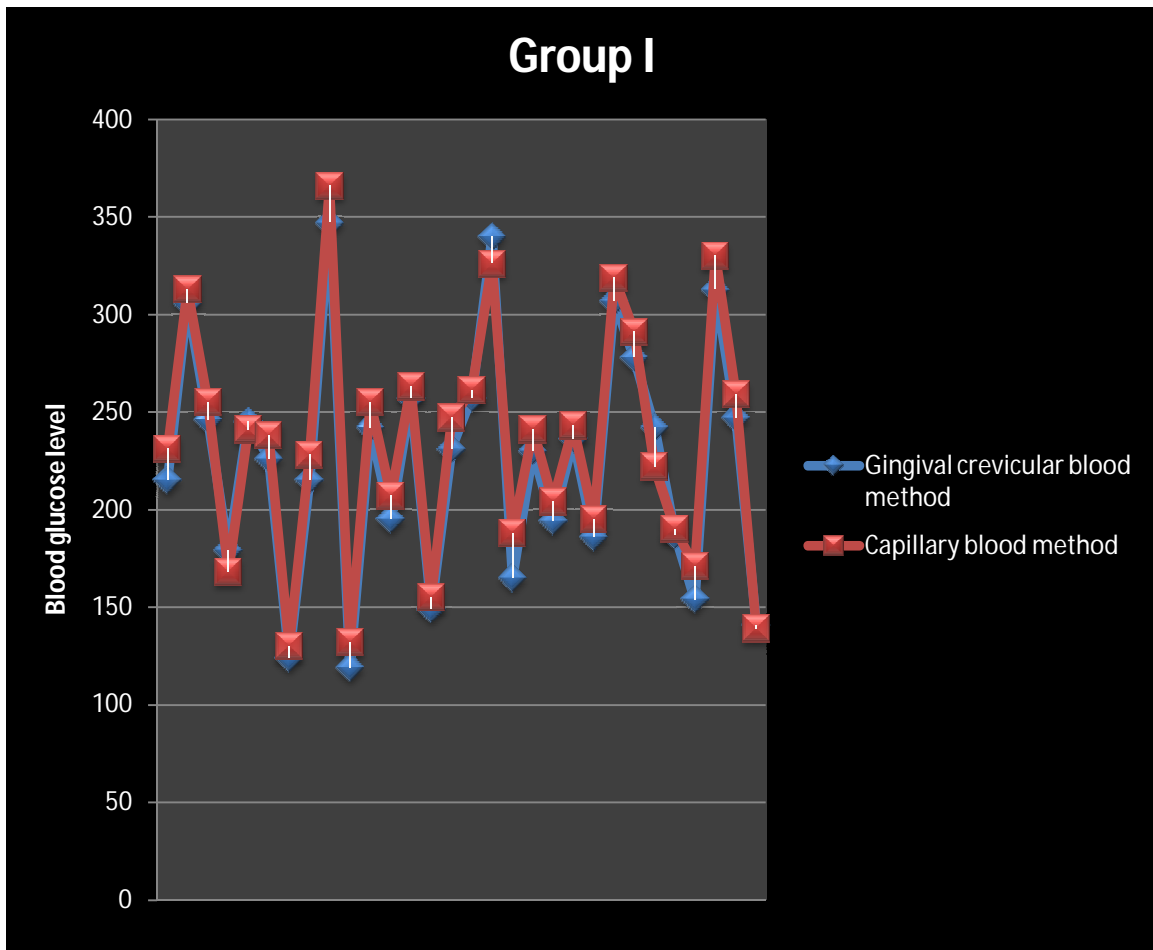


Figure 4

COMPARISON OF GINGIVAL CREVICULAR BLOOD GLUCOSE LEVEL AND CAPILLARY BLOOD GLUCOSE LEVEL IN DIABETIC SUBJECTS WITH CHRONIC PERIODONTITIS.

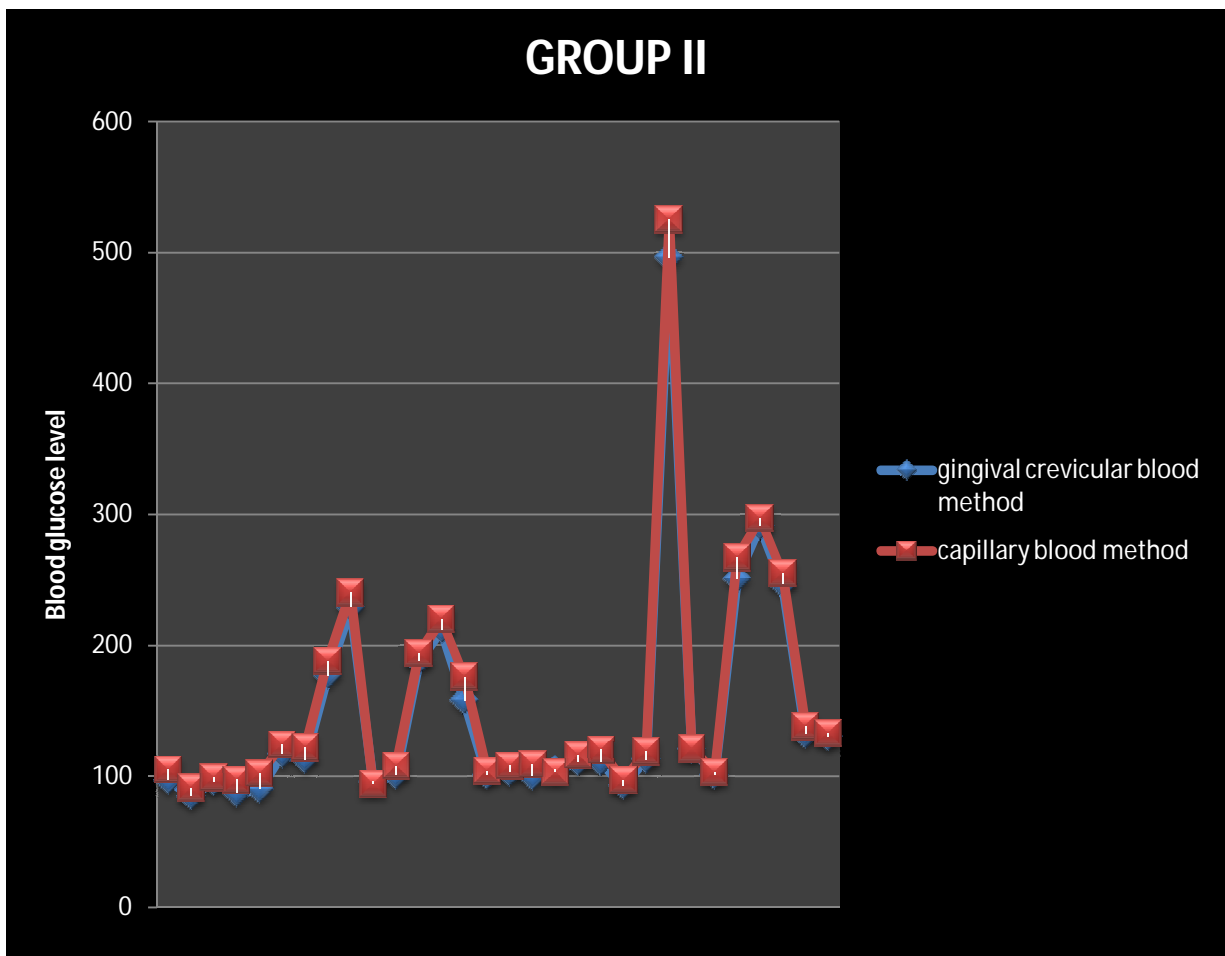


Figure 5

COMPARISON OF GINGIVAL CREVICULAR BLOOD GLUCOSE LEVEL AND CAPILLARY BLOOD GLUCOSE LEVEL IN NON-DIABETIC SUBJECTS WITH CHRONIC PERIODONTITIS.

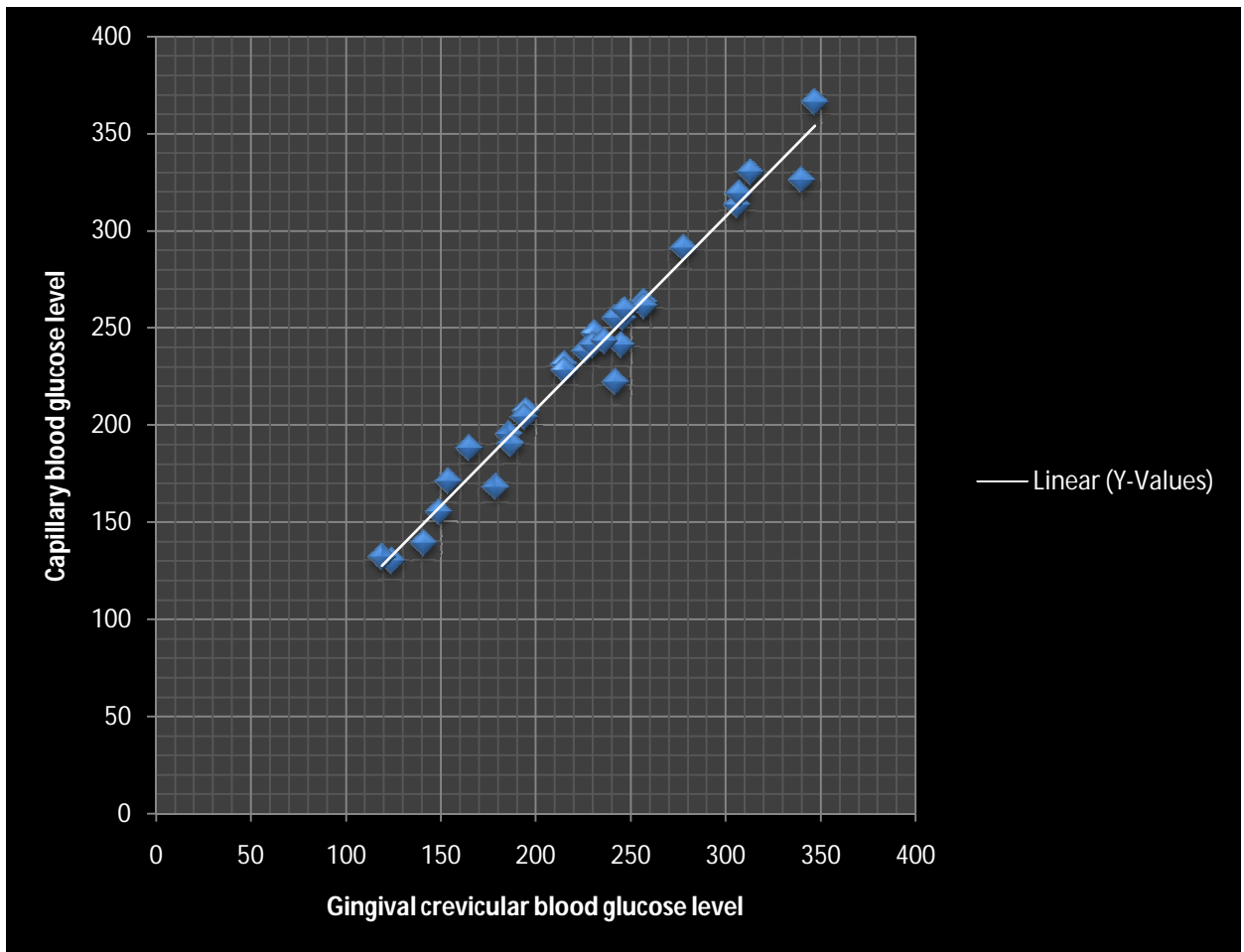


Figure 6

**SCATTERED DIAGRAM TO ESTIMATE THE CORRELATION BETWEEN
CAPILLARY BLOOD GLUCOSE LEVEL AND GINGIVAL CREVICULAR BLOOD
GLUCOSE LEVEL IN GROUP 1 SUBJECTS.**

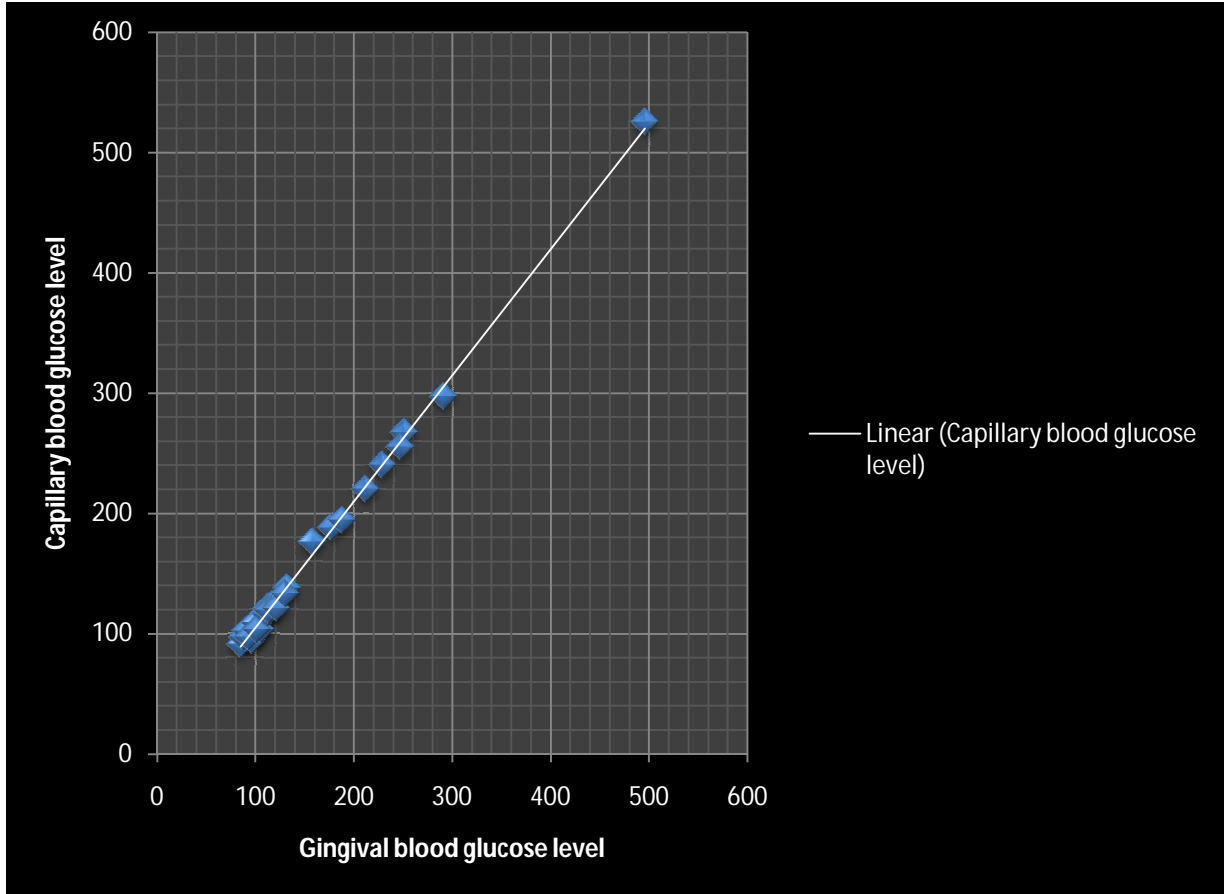


Figure 7

SCATTERED DIAGRAM TO ESTIMATE THE CORRELATION BETWEEN CAPILLARY BLOOD GLUCOSE LEVEL AND GINGIVAL CREVICULAR BLOOD GLUCOSE LEVEL IN GROUP II SUBJECTS.

DISCUSSION

Type 2 diabetes mellitus results from defects in the insulin molecule or from altered cell receptors for insulin and represents impaired insulin function i.e., insulin resistance rather than deficiency (*Atkinson MA, Maclaren NK, 1990*⁷). As the condition progresses, insulin production often decreases and patients have a relative insulin deficiency in association with peripheral insulin resistance (*Rhodes CJ, 2005*⁹²) and insulin supplementation may become necessary (*Rees TD 1994*⁸⁸). Though autoimmune destruction of β -cell does not occur, β -cell dysfunction arises from prolonged and increased secretory demand placed on them due to the insulin resistance.

In fact there is a two-way relationship between diabetes mellitus and periodontitis. On one hand, poorly controlled diabetes mellitus increases the risk for developing destructive periodontitis and impairs treatment outcome and on the other, chronic inflammatory periodontal disease considerably complicates diabetes control (*Grossi SG, Genco RJ, 1998*³⁹).

Inflammation is hypothesized to play a significant role in the development of type 2 diabetes mellitus and periodontal disease is a known inflammatory condition. Diabetic patients with periodontal infection have a greater risk of worsening glycemic control over time compared to diabetic subjects without periodontitis (*Taylor & Becker et al, 1996*¹⁰⁶).

To emphasize the problem of diabetes and to bring this problem into greater focus a general understanding of the prevalence of this disease is valuable. The increased prevalence

and severity of periodontitis, commonly seen in patients with diabetes especially with poor metabolic control led to the designation of periodontitis as the 6th complication of diabetes mellitus (*Loe H, 1993* ⁶⁶)

In the year 2000, the worldwide prevalence of type 2 diabetes was estimated to be 150 million people and it is expected to increase to 220 million by the year 2010 (*Zimmet P, 2007* ¹¹⁵). The incidence of type 2 diabetes has been reported to be increasing by up to 6% per year. (*Rees, 2000* ⁸⁹).

In 2005, India had nearly 33 million diabetic subjects with an overall prevalence rate of 4.3%. (*Ramachandran A, 2005* ⁸⁶). 70% of the current cases of diabetes occur in developing countries. With an estimated 50.8 million people living with diabetes, India has the world's largest diabetic population, followed by China with 43.2 million. (*IDF, Diabetes Atlas, 2010* ⁵⁴).

Most of the time for every 55 cases diagnosed as diabetes mellitus, it is estimated that 45 cases are completely unaware of their systemic involvement (*Stein GM, Nebbia AA, 1969* ¹⁰⁰). This estimate of potential diabetes increases in the presence of a positive family history, advanced age and stress. In the present study, 30% (i.e, 9 out of 30) of the subjects belonging to group II were newly detected with diabetes mellitus. This is in accordance with the findings of *Papapanou, 1996* ⁸¹.

Of the 9 newly diagnosed diabetes mellitus patients, 4 belong to the age group of 40 to 49 and 5 subjects were between 50 to 60 years. The incidence of periodontal disease

increases with increasing age and severe periodontal disease is associated with patients in higher age group. This is in direct correlation with the finding of *Belting et al, 1964*¹¹ who reported that the severity of periodontal disease was significantly greater among the diabetic group than among the non-diabetic group. Periodontitis and diabetes are both generally diseases of advancing age. A population with periodontal disease must be considered at a slightly higher risk of diabetes mellitus than a population without periodontal disease.

Epidemiological studies have clearly established that diabetes is a risk factor for periodontal disease and diabetes is often associated with increased gingival inflammation in response to bacterial plaque (*DePommereau et al, 1992*²⁴ & *Cianciola et al, 1982*¹⁷).

Considerable effort has been made in the past few years to develop painless and noninvasive methods to measure blood glucose in patients with diabetes mellitus so as to avoid the trauma of drawing venous blood each and every time. One of the most commonly used sites to obtain blood is from the pads of the index finger. Even though this is less traumatic than venous blood, it still is somewhat painful for the individual. Since periodontal inflammation with or without the complication factor of diabetes mellitus is known to produce ample extravasate of blood during diagnostic periodontal examination (*Ervasti et al, 1985*²⁹), no extra procedure, such as, finger puncture with a sharp lancet are required to obtain blood for glycometric analysis.

Even in the case of very low gingival crevicular bleeding, a glucose measurement is possible with the use of self-monitoring glucometer, as a very minimum amount of blood

(3µl) is sufficient to perform the analysis. The sampling procedure that was applied in this study is much easier to perform and less time-consuming, since no sophisticated armamentariums are necessary to collect gingival crevicular blood. And when comparing the glucose values between the finger prick and gingival sulcular blood, a very high level of correlation was found. In group I the correlation is $r>0.981$, $p<0.0001$ and in group 2 the correlation is $r>0.976$, $p<0.0001$ (*highly positively significantly correlated in both the groups*). This is in concurrence with *Beikler T et al, 2002* ¹⁰.

Diabetes is often associated with increased gingival inflammation in response to bacterial plaque. This response may be related to the level of glycemic control in subjects with well controlled diabetes having a similar degree of periodontitis as non-diabetic individuals and poorly controlled diabetic subjects having significantly increased inflammation. Increased gingival inflammation may be seen in diabetic subjects even though plaque levels are similar to non-diabetic controls (*DePommereau et al, 1992* ²⁴ and *Cianciola et al, 1982* ¹⁷). The level of diabetic control is a more important factor than plaque control in relation to the severity of gingival inflammation. (*Tahsin Unal, Erhan Firatli, Ahmet Sivas et al, 1993* ¹⁰⁵). Because of the ease of availability of sulcular blood, especially in inflamed periodontium, it can be used regularly to screen for diabetes.

In this present study, in group I, the mean gingival crevicular blood glucose level was lower than the mean finger-prick blood glucose level by a mean difference of 8 mg/dl. Similarly in group II also the mean gingival crevicular blood glucose level was lower than the mean finger-prick capillary blood glucose level by a mean difference of 7mg/dl. This

could be attributed to the possible contamination of gingival blood samples with saliva, plaque or gingival fluid which could dilute these samples.

Also in this present study, the intra-group correlation coefficient for group I was found to be 0.987 ($p < 0.0001$), and for group II it was found to be 0.998 ($p < 0.0001$). Hence in both the study groups the reliability of both the methods was found to be significantly high. Therefore, gingival crevicular blood can be a good alternative non-invasive source for chair-side blood glucose level monitoring.

Also the technique described is safe, easy to perform, painless and comfortable for the patient and can therefore be applied for screening of diabetes mellitus in dental offices. It takes only 5 seconds to obtain the glucose level and that too from the blood that is generated during routine periodontal examination. In addition the cost of the kit is extremely modest. Therefore the limited investment of time and money for the clinician and the patient, minimal anxiety and pain for the patient adds to the merits by which dental professionals can play a critical part in the systemic health of their patient in addition to their dental health.

Due to the close interrelationship between diabetes mellitus and periodontitis, it can be assumed that the dental practitioner and especially periodontists are extremely likely to encounter an increasing number of undiagnosed diabetes mellitus patients with periodontitis. The early diagnosis of diabetes might help to prevent the long-term complications that are responsible for the high mortality and morbidity associated with diabetic patients (*Harris & Eastman, 2000* ⁴⁵).

*Belting et al, 1964*¹¹ also concluded that the severity of the periodontal disease found among diabetics was a manifestation of peripheral vascular occlusive disorder associated with diabetes mellitus. Increased collagenase activity and decreased collagen synthesis is found in individuals with diabetes with chronic periodontitis.

AGE plays a central role in the classic complications of diabetes (*Brownlee, 1994*¹³) and may play a significant role in the progression of periodontal disease as well. In the hyperglycemic, numerous proteins and matrix molecules undergo a nonenzymatic glycosylation resulting in accumulated glycation end products (AGEs). The formation of AGEs occurs at normal glucose levels as well, but in hyperglycemic environments, AGE formation is excessive. Collagen is cross linked by AGE formation, making it less soluble and less likely to be normally repaired or replaced. As a result, collagen in the tissues of poorly controlled diabetics is aged and more susceptible to break down in periodontal tissues as well.

The cumulative effect of altered cellular response to local factors, impaired tissue integrity and altered collagen metabolism undoubtedly play a significant role in the susceptibility of individuals with diabetes to infections and destructive periodontal disease. Diabetes mellitus has a wide range of complication, such as retinopathy, nephropathy, micro and macro vascular disease, altered wound healing and periodontitis (*Ainamo J, Ainamo A, 1996*¹).

Periodontal therapy may not be associated with improved glycemic control in diabetes patients who are relatively well controlled, but may result in improved metabolic control in some individuals with poorly controlled diabetes (*Mealey 1999*⁷⁰).

Though there are a few limitations in this study such as the sample size and possible contamination of the sample with plaque, saliva, and GCF, screening for diabetes using gingival crevicular blood samples have to be encouraged as it could be the first step to identify those for whom follow-up tests regarding possible diabetes are warranted.

Because of the ease with which sulcular blood can be obtained, especially in patients with chronic periodontitis, it can be used not only to screen for diabetes mellitus, but also to monitor the level of control of the disease.

SUMMARY AND CONCLUSION

Sixty patients were screened and selected from the patients who attended the outpatient department, Department of Periodontics, Tamilnadu Government Dental College and hospital, Chennai. 30 type 2 diabetic subjects with chronic periodontitis were categorized as group I and 30 non-diabetic subjects with chronic periodontitis were categorized as group II, based on AAP classification 1999. Gingival crevicular blood and finger-prick capillary blood samples were taken to correlate the glucose levels using a self-monitoring glucometer. The data's were statistically analyzed with Mann-Whitney U-test, Wilcoxon Signed Ranks test and Spearman's Rank correlation coefficient method.

The results obtained by this study shows that both the methods are highly positively significantly correlated ($P < 0.0001$), which states that gingival crevicular blood that is expressed in a chronic periodontitis patient during routine periodontal examination can be used to screen the diabetic status of the chronic periodontitis patient. Hence the study offers an alternative method to detect diabetes in chronic periodontitis patients who were earlier unaware of their diabetic status, and would also help them to prevent developing further diabetic complications and bring down the rate of morbidity and mortality associated with diabetes.

The glucometer is a convenient, quick and inexpensive apparatus that can be used as a chair side aid during routine periodontal examination to screen blood glucose. It is easy to perform and a relatively comfortable method of obtaining blood sample.

The self-monitoring blood glucometer should not be used to replace the conventional blood glucose level measuring method. The result of the present study suggests that the gingival crevicular blood expressed during routine periodontal examination can be used to screen for diabetes.

A larger sample size and the ability to adopt the blood sample mixed with purulent discharge to determine the blood glucose level would aid in overcoming the limitations in this study and at large be of a blessing to the society.

There are several published and unpublished data's regarding the use of gingival crevicular blood to estimate blood glucose level. A meta-analysis has to be done in order to review all the published evidence to quantify the reliability of gingival crevicular blood to estimate the blood glucose level.

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APPENDIX – PROFORMA AND INFORMED CONSENT

PROFORMA

Correlation Between Levels Of Sulcular And Capillary Blood Glucose In Screening Of Diabetes Mellitus – In Chronic Periodontitis Patient

Date:	Dental O.P. No:	Code No:
Name:		Age / Sex:
Address:	Tel. no:	Mobile no:
	Occupation:	Income:

Chief Complaints:

Pain / Shaky teeth / Bleeding gums / Swollen Gums / Receding Gums / Pus Discharge / Increase in Spacing between teeth / Stains / Others.

Duration:

Medical history:

1. Diabetes Mellitus
2. Pregnancy / Lactation
3. Cardiac diseases
4. Stroke
5. Any drugs against hypercholesterolemia
6. Liver dysfunction

Dental history:

Periodontal treatment within past 6 months.

Clinical Examination:

GINGIVAL BLEEDING INDEX – AINAMO AND BAY (1975)

[illegible]

PLAQUE INDEX – SILNESS AND LOE (1964)

[illegible]

PROBING DEPTH (PD) AND CLINICAL ATTACHMENT LEVEL(CAL) (in mm)

MAXILLARY:

Palatal

CAL																
PPD																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
PPD																
CAL																

Buccal

MANDIBULAR:

Lingual

CAL																
PPD																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
PPD																
CAL																

Buccal

RADIOLOGICAL EXAMINATION :

OPG –

DIAGNOSIS :

INVESTIGATIONS:

Blood Glucose (Gingival crevicular blood) –

Blood Glucose (Finger-prick capillary blood) –

INFERENCE:

Signature of P.G student

Date:

Time:

INFORMED CONSENT FORM

STUDY TITLE:

CORRELATION BETWEEN LEVELS OF SULCULAR AND CAPILLARY BLOOD GLUCOSE IN SCREENING OF DIABETES MELLITUS IN CHRONIC PERIODONTITIS PATIENT.

Name: _____ O.P.No: _____

Address: _____ Code No: _____

Age / Sex: _____

Tel. no: _____

I, _____ age _____ years
exercising my free power of choice, hereby give my consent to be included as a participant in the study “**Correlation Between Levels Of Sulcular And Capillary Blood Glucose In Screening Of Diabetes Mellitus In Chronic Periodontitis Patients**”

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I understand that the lab investigations will require the procurement of my blood in required amount.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient

Signature / Thumb impression

Name of the investigator

Signature

Date